A CONTRIBUTION TO THE STUDY OF THE CHEMISTRY OF BLOOD SERUM.*

By ALBERT A. EPSTEIN, M.D.

(From the Laboratory of Physiological Chemistry of the Pathological Department of Mt. Sinai Hospital, New York.)

Many studies of blood serum in various diseased conditions are on record, but in summarizing the results of this work Morawitz[1] concludes that but little of importance to pathology and physiology has been gained. This is an extreme view which is not justified by a comprehensive analysis and adequate interpretation of the material at hand.

Normally blood serum varies but little in its chemical composition. Its chief ingredients, the proteins (euglobulin, pseudoglobulin, and albumin), sodium chloride, urea, and the allied nitrogenous waste products, maintain definite proportions, and may be regarded as physiological constants. Variations from the normal are known to occur in many diseases. The purpose of this work is to put on record the results of an analysis of eighty sera from different sources, and to discuss the facts elicited in conjunction with the observations made by other investigators in similar conditions.

The substances principally studied were the proteins, chlorides, and incoagulable and non-protein nitrogen.[2] Ash and total solids were also estimated in a number of sera.[3] Of the eighty sera examined, thirty-nine were from blood and forty-one from effusions of different sorts. The two groups of sera will be considered

* This work was done under tenure of the George Blumenthal Jr. Fellowship in Pathology. Received for publication, June 16, 1912.

[1] Morawitz, P., Oppenheimer's Handbuch der Biochemie des Menschen und der Tiere, Jena, 1909, ii, 79.

[2] The term incoagulable nitrogen will be used collectively in place of incoagulable and non-protein nitrogen.

[3] The sugar content of blood serum will not be discussed in this study. The nature of its variations is well understood and comprises a separate chapter in the chemistry of the blood.

separately. The present communication will deal only with the blood sera.

The sera examined were obtained from the following sources:

Normal—1 case (transfusion donor)
Diabetes insipidus—1 case
Cardiac disease—7 cases
Cardiorenal disease—5 cases
Renal disease—12 cases
Diabetes mellitus—3 cases (2 with coma and lobar pneumonia)
Pneumonia—7 cases { influenzal or tuberculous, 1; diabetic (lobar pneumonia), 2; lobar pneumonia, 4
Emphysema of the lungs—1 case
Polycythemia—1 case
Achylia gastrica—1 case

This classification is made according to the clinical diagnosis of the cases, and the diagnoses are, of course, not final. Closer pathological study with post-mortem examinations might lead to somewhat different classifications, especially of the cases of cardiac and renal diseases. To those familiar with the nature of these diseases, it is known that, as a rule, they are not essential forms. Usually cardiac diseases of the chronic type, with insufficiency, etc., are accompanied by renal disturbances or by hepatic and vascular changes. A classification of these cases into distinct pathological groups is therefore not possible. With but few exceptions the blood was obtained by phlebotomy done in the hospital routine for therapeutic purposes. This is of importance because it shows that most of the cases studied were clinically severe types, some, in fact, extreme.

According to Hammarsten and others, human blood sera show normally the following values per 100 cubic centimeters of serum:

Total protein.	Globulin.	Albumin	Incoagulable and non-protein nitrogen.	Chlorides.
5.5–8.4 gm.	37–40 %	60–63 %		0.357–0.366 gm.
Mean	38.5 %	4.2–4.4 gm.	0.020–0.050 gm.	0.361 gm.
7.0 gm.	2.7 gm.	4.3 gm.	0.035 gm.	

Ratio of globulin to albumin.
1 : 1.6

Total solids.	Ash.
8.20–9.20 gm.	0.850–0.912 gm.

One normal serum analyzed by the author gave the results shown in table I, per 100 cubic centimeters of serum. On comparing the values obtained in this analysis with the standard values given above, we find that this serum probably represents the maximum normal type. It differs from the standard in two particulars; namely, the chlorides and the incoagulable and non-protein nitrogen, both of which are high. This is probably due to the fact that the person (a transfusion donor) from whom the serum was taken partook of food shortly before the serum was removed.

TABLE I.[4]

Normal Blood Serum.

Serum No.	Total protein in gm.	Incoagulable and non-protein nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudoglobulin in gm.	Albumin in gm.	Chloride in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
38	8.300	0.062	3.071	1.059	2.012	5.09	0.397	1 : 1.7	37

Cardiac Cases.—In the analysis of the sera from the cardiac and cardiorenal cases (table II), we find considerable individual variations, some of which are quite remarkable. Four of the twelve cases (Nos. 33, 19, 23, and 36) show values for the different substances which are within the normal range of variation. Though the total protein in these cases is nearer the minimum normal amount, as determined by Hammarsten, Abderhalden, etc., the ratio of the different protein fractions to each other and to the total protein content is quite normal. A moderate state of dilution, or hydremia, would account fully for the protein values obtained. The increase observed in the amount of incoagulable nitrogen does not contradict the possibility of an existing hydremia in these cases, especially in view of the normal amount of chloride present in the sera. The increased incoagulable nitrogen might be due to a functional disturbance of the kidneys, resulting from a passive congestion.

The remaining eight cases present some interesting and instruct-

[4] In the tables the values are given in grams per 100 c.c. of serum.

ive features. With but one exception (case 51) they all show values for the total protein which are equal to or less than the normal minimum. Sera 34 and 35 are from a case of chronic myocarditis associated with chronic pulmonary edema and anasarca. The uniformity of the results of the two determinations is striking.[5] They both show a protein content which is only half the normal amount. In view of the fact that this case was associated with an anasarca, it might at once be concluded that the low protein content is the result of a severe hydremia. On analysis of all the data in this case it seems probable that in addition to the hydremia other factors are concerned in the peculiar results obtained.

TABLE II.

Blood Serum in Cardiac and Cardiorenal Cases.

Serum No.	Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
33	6.525	0.090	2.544	1.028	1.516	4.981	0.397			1:2.0	33.0
19	6.019	0.197	2.153	0.544	1.609	3.866	0.404			1:1.8	35.7
23	5.475	0.386	1.990	0.305	1.685	3.485	0.362	8.680	0.700	1:1.75	35.0
36	7.612	0.154	2.275	0.850	1.425	5.337				1:2.3	32.0
34	3.732	0.116	1.925	0.595	1.370	1.807	0.390	8.420	1.069	1:0.95	53.0
35	3.611	0.130	2.038	0.525	1.513	1.373	0.404	8.826	1.460	1:0.7	58.5
31	5.012	0.063	3.819	1.734	2.085	1.193	0.405			1:0.31	76.3
37	5.050	0.096	2.337	1.137	1.200	2.713	0.432			1:1.16	46.0
42	5.625	0.133	2.731	0.962	1.769	2.894	0.390			1:1.0	50.0
51	7.281	0.084	3.141	0.787	2.354	4.140	0.404			1:1.3	43.5
43	5.951	0.259	2.970	0.781	2.189	2.987	0.420			1:1.0	50.0
58	5.681	0.106	2.780	1.712	1.069	3.100	0.450			1:1.15	46.0

The term hydremia usually signifies a dilution of the blood; but when a simple dilution occurs, the different protein elements of the serum and plasma, which normally show definite quantitative relations, must in the diluted state retain their proportions. This, however, is not the case as the above figures for the serum fractions show. The globulins are much less reduced in amount than the albumin. In fact, the relation of the globulins to the albumin ap-

[5] An interval of three days elapsed between the removal of the two specimens of blood.

pears reversed as compared with the normal; for the globulins constitute the major part of the total protein (table II, cases 34 and 35). This shows that a simple dilution of the blood cannot have taken place, and that some other factor has contributed to this result. Just what this factor is will be explained later.

Two other features in the sera under consideration are of interest. One is that the incoagulable nitrogen as well as the chloride is practically normal in amount in the first serum (No. 34); in the second specimen, however, both values are considerably above the first, and hence, above normal. The second sample of serum shows a much higher ash content than the first. The increase noticed in the amount of total solids of the second specimen, as compared with the first, is entirely in the form of ash (Nos. 34 and 35). Although the chloride content of the second serum specimen is higher than that of the first, it does not account for the rise in the ash. A large amount of inorganic material other than chloride has therefore accumulated in the blood within three days that elapsed between the removal of the two specimens of blood. The cause of the increase in the ash content may lie in a disturbed elimination of these materials through the kidneys. The point which is of greatest importance, however, in this case, is the fact that a large quantity of inorganic material (including chlorides) evidently can accumulate in the blood, without causing a corresponding retention of water; for as the other data (i. e., the protein) of the sera show, a further dilution of the blood has not taken place, and the degree of hydremia has not increased.

The other is that the sera from the other cardiac cases (Nos. 31, 37, 42, 43, 51, and 58) also show points of interest in relation to the protein and chloride content. The protein values are again below the minimum normal. If we assume here that a dilution of the blood has taken place as in the preceding cases (Nos. 19, 23, 33, and 36) we are again confronted with the fact that the globulins bear a higher proportion to the albumin than is observed in normal sera. The chloride content of the serum is also somewhat higher than that observed normally. These two facts indicate that a simple dilution of the blood cannot have taken place in these cases.

From the above findings it is seen that the sera from cardiac cases

show striking variations from the normal. From the analysis of the blood sera, the cardiac cases are divisible into two groups: (1) One group in which a moderate dilution of the blood (a true hydremia) takes place. In the sera of these cases the proteins retain their normal proportions. The hydremia may be due to a retention of water, possibly the result of a simple functional derangement of the kidneys. (2) The other in which the proteins of the serum are moderately or markedly reduced in amount, the reduction affecting principally the albumin. The proportions of the serum proteins may be so changed that the globulins preponderate. In this group of cases there is an accompanying edema or some serous effusion. However, the change in the quantitative relations of the serum proteins is not due to a simple dilution of the blood, or hydremia.

Respiratory Cases.—These include cases of polycythemia, emphysema, and pneumonia. They are grouped together because they have one feature in common, namely, respiratory embarrassment, which, as will be shown, is of importance in the production of chemical changes in the blood.

As the figures in table III indicate, the results are of very much the same character as those obtained in the cardiac cases. The polycythemia and emphysema sera show a protein content which approaches the upper normal. In all of them, however, the globulin fraction shows a marked increase. The other data in the three sera analyzed show little of importance with the exception of the increase in the salt or chloride content.

In the four sera from the pneumonia cases, the total protein content is within the normal range. In one case (serum 41) in which the total protein falls below five grams, the decrease may be accounted for by the large amount of fibrin that was present in the blood; for, after separating the serum from the blood clot, a second clot formed. This clot was very firm, and the serum separated from the thick fibrinous mass with difficulty.

In these sera, too, we observe that the globulins give a higher value than the albumin. The sera from the two cases of diabetic pneumonia (sera 9 and 73) show a relative increase in the globulins as well, although not to the same extent as in the sera of the uncomplicated pneumonia cases.

TABLE III.

Blood Serum in Respiratory Cases.

Serum No.		Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
					Polycythemia.							
6		7.050	0.300	4.550	0.700	3.850	2.500				1:0.55	64.0
59		7.212	0.106	4.062	1.537	2.525	3.150	0.445			1:0.77	56.3
					Emphysema.							
76		6.719	0.089	3.281	1.262	2.019	3.438	0.376			1:10	50.0
					Pneumonia.							
22	Lobar	5.275	0.248	2.825	0.575	2.300	2.450	0.355	8.490	0.880	1:0.86	54.0
27	Tuberculous (?) grippe	6.588	0.118	2.370	0.700	1.670	4.218	0.376			1:1.8	35.7
41	Lobar	4.696	0.152	2.704	0.513	2.191	1.992	0.397			1:0.7	57.6
50	Lobar	6.844	0.077	3.562	1.513	2.049	3.282	0.359	9.212	0.834	1:0.9	52.6
77	Lobar	6.437	0.120	3.393	1.025	2.268	3.044	0.390			1:0.9	52.6
9	Diabetic	6.241	0.087	2.940	0.240	2.700	3.301	0.426	9.368	0.870	1:1.13	47.0
73	Diabetic	5.969	0.116	2.812	0.729	2.083	3.157	0.384			1:1.15	4.60

TABLE IV.

Blood Serum in Diabetes Mellitus.

Serum No.		Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
9	Lobar pneumonia	6.241	0.087	2.940	0.240	2.700	3.301	0.426	9.368	0.870	1:1.13	47.0
25	Pneumonia	5.625	0.116	2.537	0.635	1.902	2.088	0.460			1:0.82	54.0
73		5.969	0.116	2.812	0.729	2.083	3.157	0.384			1:1.15	46.0

The values for the incoagulable nitrogen fluctuate considerably. The amount of chloride in all the sera except one is lower than that observed in the cardiac sera. In one instance (serum 9, from a case of pneumonia in a diabetic) the chloride content is unusually high. The ash content in all the sera is uniform and practically normal in amount.

Serum 27, from a case of a typical pneumonia of undetermined etiology (tuberculosis or grippe), does not present values like those obtained in the sera of the lobar cases. The proportion of the total globulin in this serum to the albumin is below that found in normal serum. This is in conformity with the results obtained by Erben[6] in the analysis of sera from tuberculous cases.

In reviewing the results of this group of sera, we find once more that the globulin-albumin ratios are very much disturbed. The globulins in all but one case (serum 27) show a marked relative increase.

Cases of Diabetes Mellitus.—Of the three sera examined from this disease, two have already been presented in the preceding group. The serum from the third case (table IV, serum 25) gave results closely resembling those of the other two. An increase in the globulin content is again seen, which is somewhat greater than that observed in the other instances. In this case also, as in one of the other sera (case 9), there is a marked increase in the chloride content, reaching a value of 460 milligrams per 100 cubic centimeters of serum. The incoagulable nitrogen of these three sera is increased considerably over the normal amount.

Nephritic Cases.—Of this important group, twelve sera were examined. One serum from a case of diabetes insipidus is classed under the head of nephritic cases, inasmuch as it represents a type of renal disturbance. The twelve cases of clinical nephritis can be divided into three groups: (1) chronic parenchymatous, (2) chronic interstitial, (3) chronic mixed forms. Cases of acute nephritis unfortunately have not come under observation. All but two sera examined were from very severe forms of the disease, most of the cases being accompanied by symptoms of uremia, so that

[6] Erben, F., *Ztschr. f. klin. Med.*, 1903, l, 450.

the changes observed in the composition of the sera may be regarded as representative of the different types of nephritis.

The classification given above is based on clinical data and not on pathological findings. The sense which the terms parenchymatous and interstitial are intended to convey are sufficiently well understood not to require explanation. By the "mixed form" of nephritis is meant that clinical group of cases in which both the parenchymatous and the interstitial types of nephritis are, to a certain extent, represented. A contracted kidney with an intercurrent or superimposed parenchymatous inflammation may give the clinical picture of the mixed form of nephritis. On the other hand, the development of interstitial changes in kidneys affected with the parenchymatous form of the disease may lead to the same clinical result.

TABLE V.

Blood Serum in Nephritis.

Serum No.	Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudoglobulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
Parenchymatous.											
5	5.125	0.280	4.325	0.877	3.448	0.800				1:0.2	83.3
47	2.731	0.235	2.598	0.376	2.222	0.133	0.390		0.925	1:0.05	95.0
Interstitial.											
16	7 150	0.256	2.327	0.550	1.777	4.833	0.340	10.040	0.855	1:2.0	33.0
49	5.925	0.158	2.344	0.962	1.382	3.581	0.362	8.580	0.724	1:1.6	38.0
57	7.525	0.120	2.537	1.000	1.537	4.988	0.480			1:1.95	34.0
64	6.215	0.092	2.375	0.962	1.413	3.840	0.420			1:1.6	38.0
Mixed.											
24	5.656	0.073	2.375	0.415	1.960	3.281	0.532			1:1.1	41.7
39	5.300	0.133	2.187	0.450	1.737	3.113	0.397			1:1.4	41.2
48	6.800	0.151	2.837	0.956	1.881	3.963	0.426	9.920	0.870	1:1.4	41.7
55	5.644	0.085	2.800	1.181	1.619	2.844	0.362			1:1.0	50.0
62	5.202	0.085	2.371	1.234	1.137	2.831	0.412			1:1.2	45.6
65	6.775	0.116	2.875	0.912	1.963	3.900	0.390			1:1.3	42.4
Diabetes insipidus.											
78	7.919	0.011	2.645	0.700	1.945	5.274	0.302			1:2.0	33.3

In the sera from the cases of parenchymatous nephritis, we find amounts of protein which fall below the normal minimum value. In one instance (case 47) the reduction in the total protein is ex-

treme, reaching a value of about one third of the normal amount. In both cases, however, there is a marked relative increase in the globulin content, and a decrease in the albumin fraction. The increase of the globulins in one case (serum 5) is both absolute and relative. This case and the one mentioned above (No. 47) present such remarkable variations from normal that they deserve special consideration. In both instances the sera were of a greenish yellow hue and opalescent. The opalescence gave to both sera a chyliform appearance, not due to free fat, but to an increase in the lipoid content present in combination with the globulins. In serum 5 the total globulin not only constitutes the major part of the serum protein (83.3 per cent.), but is actually greater in amount than that found at any time in normal serum. The albumin of this serum, on the other hand, is diminished far below the normal, both in relative and absolute amount.

The incoagulable nitrogen in this case is also very high (0.280 of a gram in 100 cubic centimeters of serum).[7] Unfortunately an estimation of the chloride content in this instance could not be made, because of an insufficient amount of serum.

In the other case (serum 47) the total globulin is practically normal in amount, but it comprises nearly the entire quantity (95 per cent.) of protein in the serum. The amount of albumin present is so small (0.133 of a gram per 100 c.c. of serum), that it is practically negligible. As in the preceding case (serum 5) the incoagulable nitrogen is very much increased (0.235 of a gram per 100 cubic centimeters of serum), but the chloride shows absolutely no variation from the normal.

The ash in this serum is unusually high. This is of interest, especially when compared with the value obtained in the chloride estimation. This shows, as already pointed out (page 723), that the mineral content of the serum can become very much concentrated, although the relative amount of chloride remains normal.

Four blood sera were analyzed from the interstitial type of nephritis. The results in this series are fairly uniform and present

[7] Part of the incoagulable nitrogen was derived from the lipoid nitrogen present in the filtrate after coagulation of the proteins. This filtrate retained some of the opalescence observed in the original serum.

a totally different picture from the one obtained in the analysis of the sera of the cases of parenchymatous nephritis. The total amount of protein in these ranges within the normal limits; and the relation which the globulins bear to the albumin and total protein is also within the normal. There is no evidence whatever of increase of the globulin fraction; if anything, there is a tendency in these cases for the globulins to fall below normal in amount.

The incoagulable nitrogen in these sera is increased considerably above normal, but it does not attain so high a value as that found in either of the two cases of parenchymatous nephritis described above. The maximum value of the incoagulable nitrogen found in this series of cases is 0.256 of a gram, and the minimum is 0.092 of a gram per 100 cubic centimeters of serum.

The chloride content in these sera varies to a marked degree. We find here two sera with very high values for chloride, 0.420 and 0.480 of a gram, and two with moderately low figures, namely 0.340 and 0.362 of a gram per 100 cubic centimeters of serum.

The incoagulable nitrogen and chloride contents do not run parallel. In one case (serum 16) in which the incoagulable nitrogen is extremely high, 0.256 of a gram, the chloride content is very low, 0.340 of a gram per 100 cubic centimeters. On the other hand, sera 48 and 57, which do not show so great an increase in incoagulable nitrogen as case 16, show a very marked increase in the chlorides, 0.426 and 0.480 of a gram per 100 cubic centimeters, respectively.

The amount of ash in the two sera examined (Nos. 16 and 49) is below that found in any of the other cases. In both these sera the chloride content is also low. The significance of this, however, will be discussed in connection with the influence of the chemical composition of serum upon the production of nephritis.

To the group of mixed forms of nephritis belong the remaining six sera in the series given in table V. The changes which can be observed in these sera resemble in part those of the cases of parenchymatous nephritis, and in part those of interstitial forms. Of the six sera, two present normal values for the total protein; in the others, the proteins are somewhat diminished in amount. But unlike the sera of the interstitial type of cases, the ratio of the globulins to the albumin is considerably higher. This ratio, however, does

not attain the value obtained in the series of parenchymatous cases. In respect to the protein content, and the relation of the fractions to each other, the sera of these cases stand between those of the parenchymatous and the interstitial forms of nephritis.

The other ingredients of the serum also show variations which belong to one or the other group of nephritic sera. These sera (cases 65, 48, and 39) show values of 0.116, 0.151, and 0.133 of a gram for incoagulable nitrogen, whereas the other three sera (cases 55, 62, and 24) yield amounts of 0.085, 0.085, and 0.073 of a gram, respectively. Similarly the chloride content in these sera shows variations in one or the other direction, but there is no parallelism between the chloride values and those for the incoagulable nitrogen.

In some respects the results obtained in the analyses of the sera from the nephritic cases are distinctive. This is especially true of the quantitative changes in the proteins of the sera from the cases of parenchymatous nephritis. The striking variations that these sera present in this respect, as compared with those of the sera from the interstitial and mixed forms of nephritis, are of great significance for a proper understanding of the chemical pathology of kidney disease. The chlorides and incoagulable nitrogen also show marked fluctuations, but they are not as distinctive of the different sera as the changes that occur in the protein quota.

The serum from the case of diabetes insipidus presents some very interesting features. The protein content is normal or upper normal. The ratio which the globulin bears to the albumin, however, is relatively low. The globulin constitutes only 33 per cent. of the protein, a percentage like that found in the sera from the interstitial forms of nephritis. The chloride and incoagulable nitrogen show very low values, much lower than the other sera examined. The peculiar results presented by this serum are in keeping with the character of the disease itself. This will appear more clearly from the deductions to be drawn later concerning the relation which the composition of the serum bears to the clinical manifestation of the disease.

The serum from a case of achylia gastrica presents values which are normal in every respect. Clinically this case showed very little abnormality apart from the gastric disturbance.

SUMMARY.

The most striking feature observed in this study is the extensive variation of the serum in relation to its proteins. It was found that in certain diseases the globulin fraction is markedly increased, whereas the total protein of the serum may be normal, or may fall far below normal in amount. The diseases in which an increase in the globulins takes place may be grouped as follows: (1) cardiac diseases associated with decompensation and serous effusions, (2) pulmonary or respiratory affections of inflammatory or non-inflammatory origin (pneumonia, emphysema, polycythemia), (3) diabetes mellitus, and (4) parenchymatous nephritis.

In the serum of chronic parenchymatous nephritis the increase in the globulin content is most pronounced and may constitute nearly all the protein, or as much as 95 per cent.

The globulin content of serum is normal or diminished in the following diseases: (1) simple achylia gastrica (short duration), (2) tuberculosis, (3) diabetes insipidus, and (4) chronic interstitial nephritis.

Other ingredients of the sera analyzed showed variations which cannot be definitely classified; but in a general way it appears upon careful analysis that an accumulation of water and salt occurs in those diseases in which the globulin fraction of the blood serum is increased.

ON THE DIFFERENCES IN THE EFFECTS OF STIMULATION OF THE TWO VAGUS NERVES ON RATE AND CONDUCTION OF THE DOG'S HEART.*

By ALFRED E. COHN, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

PLATES 69–76.

INTRODUCTION AND HISTORICAL REVIEW.

In 1869 Meyer (1) first observed differences between the right and left vagus nerves of the tortoise as regards their action on the heart. Since that time many investigations concerning the function of the vagus nerves have been made, but the differences between the action of the two nerves have not been emphasized and have not been reported in detail.

Garrey (2), Robinson and Draper (3), and the present writer (4) only have considered that in addition to quantitative differences obtained on stimulation, essential or qualitative differences in the function of the two nerves may exist, and have studied the subject of vagus function from this point of view.

In the present communication the extensive literature dealing with the question of vagus function is reviewed in order to indicate what observations relating to differences in the action of the two vagi have already been noted. Observations from which some comparison between the action of the right and left vagus nerves may be drawn have been made on a large number of animals. In fishes and amphibia the only differences that have been noted have been differences in degree; stimulation of one nerve has been said to be more effective than stimulation of the other. Gaskell noted that in frogs stimulation of the left vagus caused blocking, but he did not stimulate the right nerve and does not describe any difference between the two nerves as regards the production of this phenomenon.

In reptiles (snake, tortoise, and crocodile) a greater effect on the

* Received for publication, June 18, 1912.

heart rate has been found to follow stimulation of the right nerve than stimulation of the left. Gaskell suggested a qualitative difference between the two nerves and stated that the "nerve fibers supplying those muscular fibers of the sinus, which by their automatic contractions originate the rhythmical beats of the heart, run almost exclusively in the right vagus." Unfortunately he did not assign any definite functions to the left vagus, and drew no definite conclusions concerning differences in function between the two nerves. Garrey, who described differences between the two nerves, ascribed to each a homolateral distribution and function. He failed to note, however, whether stimulation of one or the other vagus produced any effect on the atrioventricular bundle, similar to the effect produced by the stimulation of the left vagus nerve in dogs as described in the present communication. In birds no constant differences between the action of the two vagus nerves have been described. Only in mammals have there been observed phenomena resulting from stimulation of one or the other of the vagus nerves, which may be interpreted as definitely indicating functional differences between them. McWilliam (5), François-Franck (6), Roy and Adami (7), Bayliss and Starling (8), Rehfisch (9), Hering (10), and Rothberger and Winterberg (11, 12) have all published curves or have described phenomena occurring in their experiments which indicate such differences and which may readily be correlated with the observations made by us. In some instances the unusual effects noted by these writers followed stimulation of the left vagus nerves; in other instances the nerve stimulated is not mentioned. None of these writers, however, have made a careful study of the exact nature of the differences in function of the two nerves.

The following is a more detailed review of the literature concerning these various observations.[1]

In studying the action of the vagus in eels McWilliam (13) found that "stimulation of either the right or the left vagus is effective. And when prolonged excitation of one nerve fails to keep up the cardiac inhibition any longer, stimulation of the other vagus usually causes a further arrest of the heart's

[1] A complete bibliography and review have not been attempted. Omissions have been made, but it is believed that the present state of knowledge has been shown.

action."[2] He considered that "the ventricle is not directly influenced by stimulation of the vagus nerve,"[3] since it may be arrested but still remain quite irritable.[4]

The vagus nerves in frogs have been studied by Gaskell (14), Hofmann (15), and Guyénot (16). In Gaskell's experiments the left vagus nerve was invariably stimulated. He found that following vagus stimulation the ventricles "beat for a time synchronously with every second auricular beat."[5] In other words, he obtained an incomplete auriculoventricular dissociation. Hofmann (15) found that "the difference between the right and left vagi in the same animal consists in the fact that stimulation of the right vagus often results in the arrest of the heart, while stimulation of the left vagus, in only a weakening of the contraction."[6] These conclusions differ from those of Gaskell in that the latter considered that the left vagus has mainly a dromotropic function, while Hofmann considered the inotropic function of the nerve to be most important. Guyénot (16), while working on *Rana temporaria, Rana esculenta,* and on *Bufo vulgaris,* describes the action of the two nerves as follows: He says that (a) "in a certain number of cases the right vagus stopped the heart, while the left increased diastolic dilatation and diminished the force of the contractions without changing the number of pulsations. At other times the left was inactive, while the right did not alter the rate but increased diastolic dilatation, and left the systoles unchanged. The amplitude of the beats was increased." (b) "Sometimes stimulation of one or the other vagus nerve modified neither the number nor the force of the heart beats." (c) Stimulation of the vagus nerve often had only an accelerator or hypertonic action. McWilliam (17) studied the vagi in newts and found them somewhat different from those in frogs. For he says, "Either vagus can arrest the whole heart, but the right vagus acts much more readily and powerfully upon the ventricle than the left vagus does." The effect of the left vagus on the ventricle was not merely due to blocking, for the ventricular muscle itself had lost its irritability.

In a tortoise (*Emys lutaria*), Meyer (1) was able to produce no changes in the pulsations of the heart by stimulating the left vagus, although he could arrest the contractions of the heart with a current of the same strength applied to the right vagus. In two small specimens from Venice he also found differences between the two nerves; for stimulation of the left nerve in them slowed but did not stop the heart. Later observations on the turtle's heart were made by Gaskell (18, 19), Mills (20, 21), Kazem-Beck (22), Garrey (2), Kronecker (23), and Guyénot (24). The findings of Gaskell agreed with those of Meyer, for left vagus stimulation produced a marked alteration in force of the auricular contractions, the effect sometimes being greater than that following stimulation of the right vagus. In his complete paper Gaskell[7] (19) says of his experiments on *Testudo graeca* that stimulation of the right vagus always brought the heart to a standstill, while stimulation of the left nerve with a current of the same strength was less effectual. Even a strong stimulus applied to the left vagus did

[2] McWilliam (13), *loc. cit.,* p. 217.
[3] McWilliam (13), *loc. cit.,* p. 223.
[4] McWilliam (13), *loc. cit.,* p. 220.
[5] Gaskell (14), *loc. cit.,* p. 1012.
[6] Hofmann (15), *loc. cit.,* p. 160.
[7] Gaskell (19), *loc. cit.,* p. 82.

not affect the rate. Mills (20, 21) showed that stimulation of the right vagus nerve readily stopped the heart in *Emys lutaria,* while stimulation of the left nerve had no influence whatever;[8] in *Testudo graeca* stimulation of each vagus produced identical effects.[9] It will be seen that his results differ slightly from Meyer's and entirely from those of Gaskell. In a later paper Mills (21) reported[10] that effects of stimulation of the two vagi in the Slider terrapin (*Pseudemys rugosa*) were exactly alike in five cases, the right vagus was much more effective over the rhythm in two and slightly more effective in four cases, while the left nerve was without effect on the rhythm in two cases. He concluded that the right vagus controls the rate better than the left, though in some cases the reverse is true. The left nerve sometimes had no effect on the heart, or an effect only on its force. He added an observation[11] which was corroborated later by Garrey in a slightly modified form; i. e., that the vagi have a homolateral but never a crossed distribution. Kazem-Beck (22) in 1888 was able to arrest the heart of *Emys caspica* in seventy-five animals out of seventy-seven.[12] In the remaining two he caused a negative inotropic but no chronotropic effect. On stimulating the left vagus he caused a stoppage of the heart in forty-seven animals, a negative inotropic effect in ten, and no effect in twenty. Kronecker (23), working on *Testudo graeca,* found that "very often only the right nerve was active and stimulation of the left was without effect, just as has been described by Meyer. In many other animals the activity of both nerves was equal."[13] Of another tortoise, *Cistudo europœa,* Guyénot (25) says in his conclusions:[14] (1) Only the right pneumogastric acted on the rate of the heart. It diminished the tone of muscle in diastole, but the force of the systoles was not modified. (2) The left vagus did not act on the rate. It decreased the tone in diastole and perhaps also in systole, the force of the latter being also diminished. (3) Exceptions to these observations were found. Garrey (2) has recently shown that "the right vagus exerts a chronotropic effect, as well as other effects, upon the right veins and produces quiescence of the auricles and ventricles mainly through its chronotropic effect, rarely, with weak stimuli, the effect is brought about by production of a sino-auricular block, although in these cases the inotropic and other effects are also apparent. . . . But the left vagus decreases the excitability, conductivity, and contractility of the auricles to a point at which they fail to respond to the stimulus of the pace-maker, thus no impulses can pass through to the ventricle."[15]

Mills (26) studied the effects of stimulation of the two vagus nerves in several varieties of sea turtle and reported that "a comparison of these results with those reported for the terrapin will show a great resemblance. In by far the larger number of cases the right vagus has greater inhibitory power than the

[8] Mills (20), *loc. cit.,* p. 360.
[9] Mills (20), *loc. cit.,* p. 361.
[10] Mills (21), *loc. cit.,* p. 247.
[11] Mills (21), *loc. cit.,* p. 249.
[12] Kazem-Beck (22), *loc. cit.,* p. 348.
[13] Kronecker (23), *loc. cit.,* p. 215.
[14] Guyénot (25), *loc. cit.,* p. 1033.
[15] Garrey (2), *loc. cit.,* p. 341.

left, exceptions to this, though few, sometimes occurring. In the marine turtle no case in which the left vagus was wholly without effect on the rhythm was found."[16] He added, "I would then explain the greater effect of the right vagus, as a rule, by the character of the contraction wave, associated with the right part of the sinus and its associated veins, and by the fact that the nervous supply to this seems to be chiefly from the right vagus, rather than to any deficiency in the kind and number of the inhibitory fibers in the left vagus; both may supply an equal number of such fibers, but if the supply be even partially unilateral, then the results follow as I have endeavored to explain."[17]

In regard to snakes (*Genus tropidonotus*), Mills (27) came to the following conclusions: "(1) In no case was either vagus without effect on the rhythm of the heart. In every case actual showing, and, with a sufficiently strong current, arrest followed stimulation. (2) In the majority of instances the right vagus was more efficient than the left. (3) In a very few cases both nerves seemed to be almost, if not quite, equally influential over the heart's rate." And he adds that the vagi of the snake resemble those of other cold blooded animals he had examined.

In crocodiles Gaskell (28) found that "both right and left nerves caused an absolute and a long continued standstill even with a weak stimulus."[18]

Great variations in the results are found in the reports of stimulation of the vagi in birds. Jürgens (29) in his experiment on pigeons paid no attention to the differences between the effects of stimulation of the two nerves, but noted on his curves the nerve that had been faradized in each instance, and it is evident that stimulation of the right vagus with currents of moderate strength affected the auricles and occasionally the ventricles, but the latter, if arrested, sometimes escaped. Stimulation of the right vagus with weak currents produced only negative inotropic effects on the ventricle. Where the left vagus was stimulated, inotropic and negative chronotropic effects on both auricles and ventricles were obtained. It appears, therefore, that the ventricles sometimes were affected by stimulation of the right vagus and also, possibly more powerfully, by stimulation of the left vagus. Stübel's (30) tables showing the effects of stimulation of the vagus nerves in a variety of birds throw no light on the subject. For the purpose of the present investigation, the observations are not useful, for in many instances the two nerves were not compared in the same animal.

The action of the two vagus nerves has been studied in a variety of mammals. In the horse Arloing and Tripier (31) found[19] that stimulation of the left nerve caused a stoppage equal to the duration of three pulsations; the pulse then continued to beat with a little more force than had existed before the stimulation; during the passage of the current fifteen pulsations were counted. Stimulation of the right nerve resulted in stoppage equal to six normal pulsations, and when contractions reappeared they seemed to have great force, but only seven were counted as against fifteen during left vagus stimulation. In

[16] Mills (26), *loc. cit.*, p. 11.
[17] Mills (26), *loc. cit.*, p. 12.
[18] Gaskell (28), *loc. cit.*, p. 22.
[19] Arloing and Tripier (31), *loc. cit.*, experiment ix, p. 421.

short, stoppage was twice as long when the right nerve was stimulated as when the left was stimulated. The difference between the action of the nerves was not absolutely constant. Sometimes the usual action was reversed, and an example of this is described. Only a quantitative difference between the two nerves was found. Dissections were carried out with the view of studying the distribution of the two nerves to the heart. They found that the right vagus supplied a greater number of fibers, and on account of this preponderance they explained the greater influence that the stimulation of the right vagus has on the heart.

In cats McWilliam (5) found that on stimulation of the inhibitory structures "the ventricles sometimes give only one beat in response to every second or third auricular beat."[20] He showed further that in some cases there was ventricular standstill while the auricles went on beating. "The excitability of the ventricles, as tested by single induction shocks applied to their surface, seemed unimpaired. Moreover, mere weakness of the auricular beats would not account for the non-response of the ventricles; for very much weaker auricular beats than those observed in the above mentioned cases are quite sufficient, in the uninhibited heart, to determine the occurrence of responsive ventricular contractions. The failure of ventricular sequence was in all probability due to an interference with a mechanism of propagation quite different from the pressure changes caused by the auricular beats." Although he observed phenomena like those described in the present study as arising from stimulation of the left vagus, he did not conclude that differences existed between the two vagi, but regarded them as equally potent.

In dogs observations have been made by numerous observers, Knoll (32), Arloing and Tripier (31), Pawlow (33), Roy and Adami (7), Bayliss and Starling (8), Einthoven (34, 35), Hering (10), Dogiel (36), and Rothberger and Winterberg (11, 12).

Knoll (32) states that in three of his animals (one of which was not curarized) "there was no increase in the rate of the heart or in the height of the blood pressure after the vagi were cut." In a footnote he adds, "In one case both appeared clearly after cutting one vagus, while cutting the second contributed no added effect. As Prof. Hering tells me, this happens often in rabbits and dogs, and also stimulation of one vagus at a given strength yields an effect on the heart unequal to that of the other, even if all the conditions necessary have been observed. All this shows that in mammals, too, the inhibitory fibers of the heart are divided unequally between the two vagi."

Arloing and Tripier (31, 37) cut the medulla in a dog and found[21] that stimulation of the left vagus nerve did not stop the heart, while under the same conditions, stimulation of the right vagus led to complete arrest followed by slowing. The right nerve was more sensitive to the action of the current than the left. In two other experiments the peripheral end of the left nerve, the right nerve apparently being intact, was stimulated and the heart stopped for fifteen seconds, but contraction began again and thirty-eight of them were counted during the remaining portion of the period of stimulation. Legros and Onimus (38) attempted to show that the results of stimulating the right and

[20] McWilliam (5), *loc. cit.*, p. 189.

[21] Arloing and Tripier (31), *loc. cit.*, experiment viii. p. 421.

left pneumogastrics were too inconstant to permit the conclusion of Arloing and Tripier that differences exist between the two nerves. They based their conclusions on observations made on rats, guinea pigs, dogs, rabbits, frogs, and tortoises.

Pawlow (33) dealt chiefly with the results of stimulating the large inner branch and other chest branches of the right vagus nerve.

Roy and Adami (7), in their summary of the action of the vagus nerves in the dog, stated[22] that: "(1) With relatively weak excitation,[23] there is slowing of the rhythm but no irregularity. (2) With stronger stimulation the independent ventricular rhythm shows itself, and this, by interference with the ordinary or auricular rhythm, usually leads to irregularity of the ventricles, and, in any case, a want of complete coördination between auricles and ventricles; in other words, there is a physiological irregularity of the heart. (3) With stronger vagus excitations the auricles have either ceased to beat or the impulses which pass from them to the ventricles are too weak to excite the latter to contraction, and the ventricles are left entirely to contract by their independent intrinsic mechanism, and are completely freed from the control of the vagus, save that this nerve has still some control over their excitability." These writers did not distinguish between the effects produced by stimulating the two nerves.

Bayliss and Starling (8) obtained results similar to those of Roy and Adami, and apparently they also regarded both vagus nerves as having identical functions. In their experiments the right nerve apparently remained intact and the left one was cut. They stimulated with strong currents, and "thought[24] that the auricles had entirely ceased beating and that the ventricles were beating with their own proper rhythm." They consider it "possible that an excitatory process may occur in the auricle and may be transmitted thence to the ventricle without causing any appreciable (i. e., registrable) contraction of the auricle itself." Further on in their paper they made the observation[25] that stimulation of the vagus can cause incomplete heart-block when the ventricles "only respond every now and then to an auricular beat."

Einthoven (34, 35) studied with the string galvanometer the results of stimulating the vagus nerves. In his first paper (34) he has reproduced a curve of right vagus stimulation (figure 33) and another curve (figure 34) which resembles those we have obtained following stimulation of the left nerve, but he does not mention which nerve was stimulated. His later paper (35) added no further data on the differences between the two nerves.

Hering (10) has published curves (figures 5, 6, 7, and 8) showing ventricular silences following vagus stimulation; that is, there was incomplete heart-block, usually at the ratio of 2:1. One of his curves (figure 5) indicates the occurrence of a high degree of block, but this occurred only when the left vagus nerve was stimulated.

Dogiel (36) states that[26] "many physiologists have called attention to the fact that stimulation of the right vagus has not the same effect on the heart

[22] Roy and Adami (7), *loc. cit.*, p. 237.

[23] Of the vagus nerve in the dog.

[24] Bayliss and Starling (8), *loc. cit.*, p. 410.

[25] Bayliss and Starling (8), *loc. cit.*, p. 412.

[26] Dogiel (36), *loc. cit.*, p. 122.

action as stimulation of the left, a result due in part to the strength and duration of the stimulus."

Rothberger and Winterberg (11), in one experiment which they have described in detail, observed[27] that "the effect of a simple stimulation of the two vagi differed in so far that stimulation of the right caused complete stoppage, while stimulation of the left vagus caused only slowing of the auricular contractions with complete cessation of the ventricular (figure 7 b)." Their comment on the result obtained in this experiment is[28] that "exceptionally the inhibitory nerves for the sinus node pass exclusively in the right, for the atrioventricular groove, on the other hand, in the left vagus." And finally they state,[29] "Inhibitory fibers for the left auricle and the atrioventricular groove are usually so divided that inhibitory effects can be obtained by stimulating either vagus. Only in a few cases do only negative chronotropic fibers for the primary pace-maker pass in the right vagus."

François-Franck (6) states[30] that he has seen that "stimulation of the vagus nerve can suppress ventricular while auricular contractions continue. To bring about this result induced currents were used, weak enough not to cause complete arrest of the whole heart. These stimuli, for example, slowed the ventricular beats by five, while they caused the auricles to lose only two systoles in five. Three auricular beats occurred then during a long ventricular pause." The result is like that due to stimulation of the left vagus, which is described in this paper.

Hunt and Harrington (39) were able to produce incomplete heart-block in the calf by stimulation of the vagus nerve, but they fail to mention which one was used.

Before the publication of the work of Mills and Garrey, Masoin (40) in 1872 seems to have been the only experimenter who had insisted that this question of difference between the vagi involved that of the essential asymmetry of the body as far as the vagus nerves are concerned. In his experiments on rabbits he found that stimulation of the right nerve was more effectual in slowing the rate of the heart than stimulation of the left. He states, however, that the two nerves have no special sphere of action and so denied the existence of qualitative differences between them.

The vagus nerves in rabbits have been studied by Langendorff (41), Johansson and Tigerstedt (42), and by Rehfisch (9). Langendorff (41) experimented on sixteen rabbits. In three animals stimulation of each vagus had an equal effect on the heart; in three animals the right vagus was more effectual; in eight, the left; and in two sometimes one and sometimes the other was more effectual. The difference in effect of the two nerves was determined by the degree of slowing produced. Johansson and Tigerstedt (42) working on rabbits confirmed the work of McWilliam (5) on cats. They stimulated only the left vagus nerve and did not register the auricular contractions. They found that weak currents only slowed the ventricles (and the auricles), but that currents

[27] Rothberger and Winterberg (11), *loc. cit.*, p. 356.
[28] Rothberger and Winterberg (11), *loc. cit.*, p. 361.
[29] Rothberger and Winterberg (11), *loc. cit.*, p. 372.
[30] François-Franck (6), *loc. cit.*, p. 403.

of increasing strength not only slowed but decreased the strength of ventricular contractions until they ceased to beat. Rehfisch (9) found[31] that "a fifth effect of vagus stimulation* is a single or succession of ventricular silences, while the auricles continue to beat (see figure 5)." Two explanations were offered for this phenomenon. The first explanation, that it was the result of negative dromotropic effects on the A-V bundle, was rejected because there was no delay in the As-Vs interval, either in the cycles which preceded or in the cycles which followed the ventricular silence. He favored a second explanation; namely, that there was either a diminished strength in the conduction impulse or a diminished irritability in the ventricular or the A-V systems.

In guinea pigs Harrington (43) found[32] both vagi to be always efficient and in no case was he able to discover any greater efficiency of one than of the other nerve.

The action of the two vagus nerves in the human subject has also been investigated. The method of investigation has consisted in exercising digital pressure along the course of the intact nerve in the neck. This method was described by Czermak in 1865 and its value has been demonstrated repeatedly since then. This method was employed by Robinson and Draper (3) in a comparison of the action of the two nerves in man, and they found that differences similar to those about to be described in this paper could be elicited. It must be stated that the method is uncertain, and uniform results cannot always be expected.

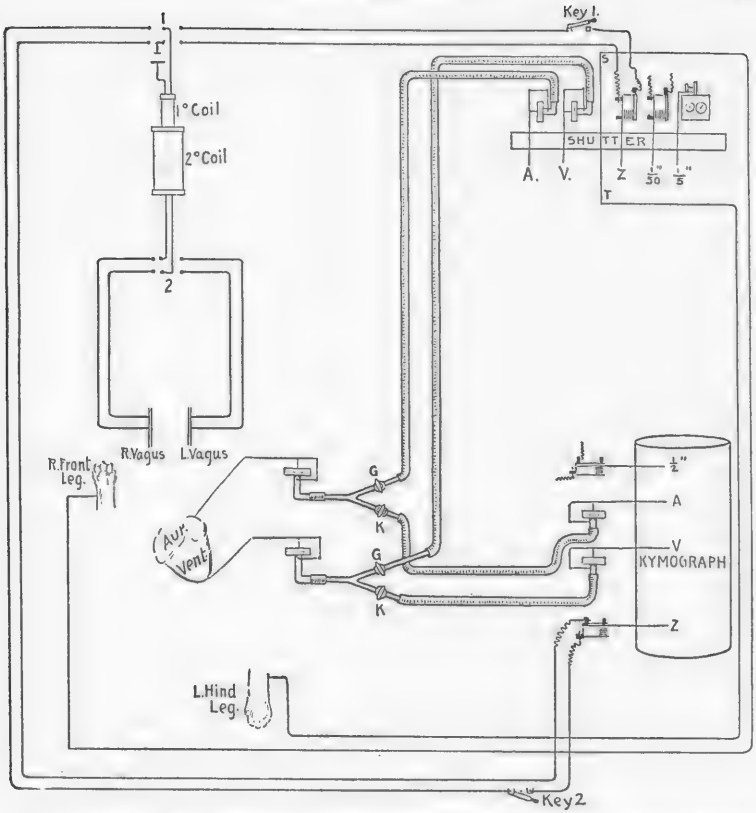
METHODS.

In this investigation only dogs of medium size were used, weighing usually from 5 to 7 kilos; a few weighed from 10 to 12 kilos. The dogs were first anesthetized with ether in the ordinary way; no other drugs were used. When complete anesthesia had been established, a catheter was introduced through the mouth, larynx, and trachea, after the manner described by Meltzer and Auer, and through this air and ether, in regulated quantities, were administered. The pressure in the tube just before it entered the mouth was read on a mercury manometer which was connected with the catheter by a side arm. A pressure of about 20 mm. of mercury was maintained. When the chest was opened, if the animal was well anesthetized, the lungs appeared in a semicollapsed condition, but they evidently contained sufficient air, for even after operations lasting four to five hours the animals were in excellent condition. The fact that the lungs were collapsed and practically motionless facilitated the use of a suspension apparatus to register the motion of both auricles and ventricles. This was accomplished in the usual way by means of hooks from the right auricle and right or left ventricle, or by means of Roy and Adami myocardiographs leading to two receiving tambours, which were connected with writing tambours by air transmission. The writing tambours were four in number, two arranged to write on a Reickert kymograph and two hung before the shutter of a photograph box. One pair or the other could be set in motion by opening and closing stopcocks in two Y tubes (text-figure 1), placed, one each, in the transmitting tubes

[31] Rehfisch (9), *loc. cit.*, p. 170.

* Single induction shocks were used.

[32] Harrington (43), *loc. cit.*, p. 393.



TEXT-FIG. 1. The connection of the dog with the recording apparatus is shown. The auricles, ventricles, two vagus nerves, right fore, and left hind leg are indicated. Registration on the kymograph is effected when the stop-cocks G are closed and the stop-cocks K are opened, and when the double throw-switch 1 is thrown to the right. Right and left vagi are stimulated according to whether double throw-switch 2 is thrown to right or left. Registration is effected on sensitive paper behind the "shutter" of the photograph box, when stop-cocks K are shut and stop-cocks G are opened and when the double throw-switch 1 is thrown to the left. Keys 1 and 2 complete the circuit by which stimulation of the nerves is effected. A signal, Z, indicates the duration of the stimulus. A and V represent the tambours registering the motions of the auricle and ventricle, one each before the shutter and before the kymograph. A time-marker recording one half seconds was employed for the kymograph; two time-markers recording 0.02 of a second interruptions made by a tuning fork and 0.2 of a second made by a Jacquet clock were employed for the photographic records. The right fore and the left hind leg are shown connected with the string, ST, of the galvanometer.

from auricles and ventricles. On the kymograph, besides the motions of auricles and ventricles, was written the time in one half seconds and the record of faradic stimulation by a signal in connection with the primary coil of a Harvard inductorium. The primary coil was supplied by a dry cell, known always to have a potential of two volts and an amperage not below twelve. In a number of experiments in the beginning the suspension methods alone were used. Later curves were made with a string galvanometer (Edelmann's larger model). By means of the device already mentioned, the tambours hung before the shutter of the photograph box could be set in motion, and the shadows thrown by their writing points were photographed on sensitive paper together with the oscillations of the galvanometer string. In many experiments only galvanometric records were obtained. Three kinds of electrodes were used to connect the dogs with the galvanometer. At first those devised by Gotch for taking direct leads from the heart were employed. Although electrodes of this form may attain much flexibility and usually follow the motions of the heart well, they were soon discarded for two reasons. First, the rapid motions of the hearts of dogs make it very difficult for the worsted threads to maintain satisfactory contacts with the surface of the uninjured heart, and owing to these motions distortions of the curves frequently occurred and sometimes interfered with their interpretation. In the second place, the curves thus obtained differed so much, both in size and shape, from those with which we are familiar that their interpretation was difficult. The second form of electrode used was that recommended by Einthoven for dogs. The legs were wrapped with gauze saturated with salt solution, the end of the gauze lying in a vessel of salt solution. In this solution a porous vessel containing zinc sulphate solution was placed. From a zinc plate in the latter solution, copper wires led to the galvanometer. The third form of electrode used was the simplest. It consisted of plates of German silver wrapped on both sides with felt or cotton saturated with salt solution. These were placed around the legs of the animal. Binding posts soldered into the German silver plate formed the point of connection between the electrodes and the galvanometer. The second lead (right fore leg and left hind leg) was used in all these experiments. Curves have been obtained with the chest unopened and also opened, and by direct and indirect leads. It may be stated that for the purposes of this investigation the results did not differ with the different methods.

The greatest amount of information was obtained when the galvanometric and the suspension methods were combined. The diminution in the muscular activity of the heart which takes place during stimulation of the vagus nerves is frequently so great that an inspection of suspension curves or of curves of muscular shortening leads to the belief that all cardiac activity has ceased. During such periods, however, it is quite apparent that cardiac activity may, and in fact does often continue and causes distinct and sometimes large oscillations of the string of the galvanometer. Without electrical tracings certain of the experiments of this series would not have yielded the results which are now presented, and some of the general rules deduced could not otherwise be stated. On account of the relatively slight value, therefore, of suspension curves and on account of the complications due to an extensive operation on the chest, the results of a number of the experiments are based on galvanometric curves only.

The experiments in which only suspension curves were obtained or in which the galvanometric curves were unsatisfactory have been discarded.

The curves made by the oscillations of the galvanometer string were photographed directly on bromide paper. Above the curves showing the movements of the galvanometer string there were photographed the movements of the tambour levers recording auricular and ventricular contractions, and below it, first a signal indicating the period of stimulation of the vagus nerve, then the rapid motions of a tuning fork, a double motion of which equals 0.02 of a second, and finally a curve the peaks of which show the movements of the lever of a Jacquet clock 0.2 of a second apart. Whenever possible, both methods of marking time were employed to facilitate subsequent measuring. The photographed curves then show from above downward (1) the curves of the auricular and (2) of ventricular contraction, (3) the curve of the galvanometer string, (4) the stimulation signal, (5) a time curve in 0.02 of a second, and (6) a time curve in 0.2 of a second.

In every experiment both vagosympathetic nerves were dissected without injury, as far as this was possible, and each was laid and secured in a shield electrode. To prevent a spread of the stimulating current the nerves and shield electrodes were surrounded by a rubber membrane which was kept dry by laying it on a pad of gauze placed in the groove occupied by the carotid sheath. The muscles and skin were then drawn over the nerves. In most instances spread of the current was satisfactorily prevented and the nerves remained in functional activity throughout the period of the operation.

Curves were always taken either on the kymograph or photographically before opening the chest and also when the chest was opened and before section of the vagus nerves. Sometimes curves were taken before and after the section of each vagus nerve. A number of curves were usually taken showing the effects on the heart of faradic stimulation of each vagus nerve. The curves were so taken that they included the heart action before stimulation, as well as that during the entire period of stimulation, and they were continued until the heart returned to a condition approximately the same as that before stimulation. The first portion of each curve serves as a control.

The strength of the stimulating current was usually the same. One two volt cell fed the primary coil of the inductorium and the secondary coil was in almost all the experiments placed fifty millimeters from the primary coil. Exceptions to this rule are noted where they occurred. In a given case the conditions of stimulation throughout the experiment were kept constant, so that inequalities from this source cannot be held responsible for the differences in effect which have been observed. It is conceivable that, were the strengths of the current used on the two sides varied, identical results on stimulation might have been obtained. It is believed, however, that enough evidence is at hand to show that such would not be the case. A report bearing on this point will be made later.

RESULTS.

In the course of this investigation fifty-four experiments were performed. Differences in the action of the two nerves were found in forty-eight (88 per cent.) of the dogs. Stimulation of the right vagus nerve arrested all portions of the heart in fifty-one experiments. These observations do not differ from

those with which we are familiar. In most instances arrest of the whole heart, after a latent period of 0.4 of a second, was maintained throughout the period of stimulation, the duration of which was sometimes ten seconds, but usually five to eight seconds. Occasionally, however, the auricles and also the ventricles, after having been arrested, escaped from inhibitory control (nine experiments). When the ventricular complexes in the cycles which escaped were normal, that is to say, when they were identical with those recorded before the beginning of stimulation, they were preceded by auricular contractions, as evidenced by the presence of P waves. In these cases the whole heart or its pace-maker escaped inhibition. But there were other cases in which, during stimulation of the right vagus nerve, ventricular complexes appeared which differed from those seen before the beginning of stimulation (experiment 639). These differences were not differences of degree, they were differences in kind.[33] When these complexes occurred during stimulation of the right vagus nerve, waves representing auricular contractions were absent. At the end of the period of stimulation, or sometimes before the end, when the pace-maker resumed its function, the abnormal ventricular complexes gave way to normal ones; the usual As-Vs sequence was restored. These abnormal complexes have been interpreted by their configuration as representing cardiac contractions originating in some infra-auricular portion of the heart, either in the right or in the left ventricular muscle. Such complexes are seen in certain forms of poisoning when the pace of the heart is determined by the ventricular contractions. They are evidence of increased irritability of the muscle of the ventricles and appear when idioventricular contractions due to the increased irritability of the muscle occur during the period of stimulation. It is questionable whether the occurrence of abnormal ventricular complexes, representing a new form of cardiac activity, should be considered as evidence that the ventricular muscle has escaped from the inhibitory control of the vagus nerve. It is, on the contrary, doubtful whether ectopic ventricular contractions can ever be subject to inhibitory action.[34] From these experiments it may be asserted that stimulation of the right vagus nerve causes arrest or rarely only slowing of the whole heart. In the latter event the whole heart beats in a normal (nomotopic) fashion. It is apparent that if the movements of the auricles and the ventricles had been registered by the suspension method alone, the nature of the idioventricular contractions could not have been determined. In experiments by others in which ventricular contractions during right vagus stimulation have been registered by the suspension method, these contractions have been considered to indicate a failure of vagus inhibition of the ventricles. Possibly they belong to the group which has just been discussed.

Stimulation of the left vagus nerve in the majority of cases did not stop the heart. The heart continued to contract, but some form of disorder in the sequence of contraction took place. In addition a diminution in rate occurred. Both effects were constant. The latter effect may be regarded as a quantitative difference in the action of the left vagus from that of the right, but the former did not occur when the right vagus was stimulated and indicates a qualitative difference. Further attention has not been paid in this study to the negative

[33] It is not possible within the limits of this paper to discuss purely electrocardiographic phenomena. It is believed that interpretation of the latter has been sufficiently established to permit conclusions to be drawn from them.

[34] Experiments dealing with this phase of the subject are in progress.

chronotropic action of the left vagus. The nature of this action is still obscure. The present study deals mainly with the dromotropic effects of stimulation.

The hearts of all dogs that were experimented upon were not influenced to the same extent by stimulation of the left vagus nerve, but whenever the normal rhythm was changed, the change which occurred was always of the same character and varied in degree only. It is useful to divide the experiments into groups which exhibit effects ranging from the simplest to the more complicated.

In the first group (table I), in which are included eight experiments, stimulation of the left vagus nerve, aside from slowing the heart, caused only a delay in the time of conduction from auricles to ventricles. There was no disturbance in the auriculo-ventricular sequence, there was no change in the form of the ventricular complex. Changes in the As-Vs conduction time also sometimes occurred during stimulation of the right vagus, but they were never so marked or so constant as those resulting from left vagus stimulation and occurred only in isolated escaped cycles, or for one or two cycles after the cessation of stimulation. Moreover, these slight disturbances in conduction following right vagus stimulation were associated with other phenomena characteristic of right vagus stimulation. The first group, therefore, comprises those experiments in which stimulation of the right vagus nerve arrested the motions of the heart, while stimulation of the left merely slowed them and caused a marked delay in the time of conduction.

The second group includes twenty-four experiments in which on left vagus stimulation some auricular contractions failed to inaugurate ventricular beats. In most instances a ventricular contraction followed every second auricular contraction, but occasionally three, four, or more auricular beats occurred before ventricular contraction occurred. Sometimes these forms were combined and occasionally during a stretch of one or another form a normal As-Vs cycle was interposed. Stimulation of the right vagus nerve arrested the motions of the heart as usual.

In the third group, in which are included twenty-four experiments, the heart behaved in the usual way when the right vagus nerve was stimulated, but when the left vagus was stimulated, nomotopic ventricular contractions ceased entirely, while auricular contractions continued, though at a slower rate. The mechanism of ventricular asystole in these cases is difficult to explain at present, except on the assumption that the ability of the structures of the A-V system to conduct impulses was enormously depressed and that the irritability of the ventricles was not sufficient for them to develop a rhythm of their own, at least within the time occupied by stimulation. In certain cases (experiment 639) during stimulation of the left vagus, ectopic ventricular contractions occurred in complete dissociation from the slowed auricular beats. It must be assumed that in these instances the irritability of the ventricular muscle was sufficiently great to initiate an independent rhythm during the period of stimulation. In some cases only a few contractions, representing the beginning of an independent ventricular rhythm, occurred during stimulation.

DESCRIPTION OF CURVES.

Stimulation of the right vagus nerve in the greater number (fifty-one) of experiments arrested all portions of the heart. Figure 1, experiment 673, illus-

trates this effect. The stimulus lasted for 6.5 seconds. The whole heart ceased to beat during the period of stimulation and for about 1.2 seconds longer. The latent period was 0.4 of a second, about equal to the duration of a cardiac cycle. The As-Vs (P-R) interval before stimulation equalled 0.09 of a second, and for the first four cycles after stimulation lengthened to 0.1, 0.11, 0.12 and 0.11 of a second. The P wave was small at first, but later became diphasic. This phenomenon has been described by Einthoven.

Numerous differences in detail from the curve (figure 1) which has been taken as characteristic of the result of right vagus stimulation were noticed. Occasionally the P-R interval remained unchanged after the cessation of the stimulus, while in some experiments it lengthened as much as 0.05 of a second. In other instances, an auricular contraction, that is to say a P wave, not followed by a ventricular contraction, occurred after the stimulus began to be effectual. An instance of this is shown in figure 2, experiment 666. In the same curve two complete cycles appear during stimulation of the right vagus nerve, and between the two a blocked auricular contraction, P′, is seen. The appearance of a blocked auricular systole in this situation during stimulation was relatively rare. The P-R intervals lengthened during the period of stimulation from 0.1 to 0.12 and 0.14, but afterward they fell again to 0.11 and 0.1 of a second. A blocked auricular systole, P′, is shown in figure 3, experiment 666, but here it occurs after the cessation of stimulation following the first returning cardiac cycle. A blocked auricular systole in this situation was not infrequent. A slight increase (0.1 to 0.12) in the P-R time occurs in this curve. After stimulation the R waves increased in size.

It has been mentioned that during stimulation of the right vagus nerve, the ventricles sometimes continued to contract, although the auricles had ceased. When this occurred, the origin of the ventricular contractions was ectopic. These escaped ventricular contractions differ, therefore, from the ventricular contraction seen in figure 2. Sometimes only a few ventricular contractions appeared, but occasionally a relatively rapid idioventricular rhythm arose. In figure 4, for instance, experiment 659, only two ectopic ventricular contractions escaped at irregular intervals during a period of stimulation lasting 6.5 seconds. The complexes correspond to contractions originating in the right ventricle. This curve shows again the occurrence of a blocked P′ wave after the beginning of effective stimulation, and also blocked P′ waves after the end of stimulation. The increase in the P-R time in the first returning cycle increased more than was usual, 0.1 to 0.18 of a second. In figure 5, experiment 661; ectopic ventricular complexes appear, the result in this instance of contractions arising in the wall of the left ventricle. These occur at fairly regular intervals and develop a rapid rate, sixty per minute. No evidence of auricular contraction can be seen during the period of stimulation, but at its cessation, three P waves appear before a nomotopic ventricular complex occurs. The P-R time is slightly increased, 0.11 to 0.14 of a second. The P waves have a tendency to be diphasic. It is not quite clear why the first returning P wave fails to be followed by a nomotopic R wave. The explanation probably is to be found in the fact that conductivity was much depressed and the impulse reached the ventricular muscle after the ectopic pace-maker had discharged its stimulus, and P′ found the ventricle refractory. In figure 6, experiment 639, precisely similar events are

shown as in figure 5, the difference between the two consisting in the fact that the ventricular complexes originated in the right ventricle and beat at a rate of 92.4 per minute.

An effect which resulted from stimulating the right vagus and which was seen only once in this series of experiments (experiment 625) consisted in an unusual slowing of both auricles and ventricles. They were slowed not in the usual way in which the sequence of contraction is maintained, but for three cycles during the period of stimulation, the P waves fell between the R and T waves. The R-P interval was too short to permit the interpretation that a reversal (Vs-As) of the normal rhythm had probably occurred; the unusual sequences probably resulted rather from the fact that both auricles and ventricles received their impulses from the junctional tissues lying between them and the course of the stimulus flowed upward and downward to each. Direct leads were used in this experiment so that deductions are not drawn from the form of complexes found, although the form of the P wave was altered during the period of stimulation.

The simplest effects on stimulating the left vagus nerve are seen in figure 7, experiment 662. During a period of stimulation lasting 7.8 seconds there occurred, first, a reduction in rate from 135 to 114.2 beats per minute, and then, an increase in the time of conduction from auricle to ventricle (P-R interval) from 0.1 to 0.24 of a second. The P-R time lengthened in general from the beginning to the end of stimulation. Toward the end of stimulation, the P waves tended to become diphasic, an effect noticed also on stimulating the right vagus nerve. A small positive wave before and immediately connected with the P wave also appeared during the time of stimulation. The split P wave has been noticed[35] by Hering (10). In view of the general dissociative effects described in this study as resulting from stimulation of the left vagus nerve, some degree of contributory evidence is lent to Hering's surmise that this wave represents a succession in the contraction of the right and left auricles. It is to be noticed that the effect of the stimulus lasted more than 3.5 seconds after its cessation.

A more complicated effect which results from left vagus stimulation is illustrated in figure 8, A and B, experiment 666. The rate of the whole heart was reduced from 173.6 to 113.2. The slowing was progressive, as can be demonstrated by the increased time between the auricular contractions, the P-P waves. Before the application of the stimulus, this interval was 0.35 of a second, but it gradually lengthened to 0.55 of a second. About two seconds after the end of stimulation the P-P interval was normal again. A further effect consists in the occurrence of blocked auricular contractions (P′) during the period of stimulation. In figure 8, A and B, six blocked P′ waves are seen. The P-R interval in this curve, as in digitalis poisoning, gradually increased until an auricular wave (P′) failed to be followed by an R wave. Progressive increase in P-R time of this nature occurred in the first three groups of cardiac cycles, but in the fourth the progression was not uniform (0.11, 0.15, 0.14, 0.17, and 0.14 of a second). As the length of the stimulation increased, all the P waves became diphasic.

Finally the most marked effect that is seen as the result of stimulating the left vagus nerve is illustrated in figure 9, experiment 673. During a period of stimulation lasting 7.2 seconds, the auricular contractions continued uninter-

[35] Hering (10), *loc. cit.*, p. 158.

ruptedly, but the rate was reduced from 176.64 to 79.56 per minute. The ventricular contractions, however, ceased for nine seconds; that is to say, throughout the period of stimulation and for some time after it. The first ventricular contraction to return after the end of the stimulus did so two seconds after stimulation had ended and 0.12 of a second after the preceding P wave. The P′ which follows the ventricular beat was blocked, but the sequential contractions of the heart were then resumed. The P-R interval became longer, 0.14 of a second, than it had been before stimulation, but it gradually returned to normal. The R waves increased in size from six to eight millimeters for six cycles and then suddenly returned to the original height. The curve also shows that during stimulation seven or possibly eight waves can be identified in the suspension curve of the right auricle. These become progressively smaller and finally disappear. The corresponding P waves in the electrocardiogram, on the contrary, become a little larger near the end of stimulation and can, in fact, be identified without difficulty. It is this gradual disappearance of the waves of the suspension curve, while the electrical ones remain, which made it necessary to discard those experiments in which only the former were made.

Another example of this degree of effect of left vagus stimulation is seen in figure 10, A and B, experiment 667. Except for the preliminary arrest of the auricular (2.3 seconds), as well as of the ventricular contractions, the results of stimulating the left vagus were similar to those seen in figure 9. The auricular rate was about 96. After the cessation of stimulation, its effect continued in a form previously described. At first one ventricular contraction took place long (0.48 of a second) after the auricular beat preceding it. This ventricular beat was probably ectopic, especially since its size is greater than that of the other complexes. A ventricular beat did not appear again until five more auricular contractions had occurred. A succession of sixteen ventricular contractions followed this one, each preceded by two auricular beats, producing incomplete heart-block at a ratio of 2:1. One sequential cycle and another ventricular contraction following two auricular systoles followed, when the normal rhythm was finally resumed. The P-R interval was 0.12 of a second before stimulation; it reached 0.2 of a second during the period of incomplete block, and twenty seconds after stimulation it was still 0.16 of a second. So great an after effect, lasting so long a period, was observed only in this experiment.

In the experiments just described, complete ventricular asystole was present throughout the period of stimulation. But in a number of experiments, ventricular quiescence did not take place. Normal ventricular complexes disappeared and one must conclude that normal ventricular contractions had ceased. The complexes representing the ventricular beats which continued have the form of those arising in the wall of the right or the left ventricles, and are assumed to be ectopic in origin. They were the result of an idioventricular rhythm arising because impulses failed to reach the ventricles on account of the depression in conductivity of the auriculoventricular system during stimulation of the left vagus nerve. In so far as the ventricles under these circumstances did not receive supraventricular impulses, they are comparable to experiments on auriculoventricular block.[30] Figure 11, experiment 639, illustrates this reaction

[30] The relation of the two vagus nerves to heart-block after it has been established is not discussed in the present communication.

to stimulation of the left vagus nerve. The stimulus lasted 6.3 seconds. During this time the auricular beats continued at a rate of about 127. The usual ventricular complexes changed to a new form, which was maintained during stimulation and for one second afterwards. One second was about the average length of time that the effect continued after stimulation had ceased. The rate of the ectopic ventricular beats was about 86. The curve shows that when the ventricular contractions were ectopic, they occurred in complete dissociation from those of the auricles. Suspension curves alone would only have disclosed the fact that dissociation between auricular and ventricular contractions had taken place. But the electrical curves make it clear that dissociation in the usual sense did not occur, for in that event the form of the ventricular complexes would not have been altered. An explanation of why they are changed is difficult and the hypothesis which follows is offered tentatively. In cases of clinical heart-block the anatomical lesion, which is the cause of the block, usually involves only a short portion of the main stem of the conduction bundle; the right and left branches are normal, and are left in continuity with the ventricular muscle. The ventricular muscle continues to receive impulses to contraction propagated to it apparently over the usual course provided by the two branches. But there are cases, both clinical and experimental, published by Eppinger and Rothberger (44), Eppinger and Stoerck (45), and by Cohn and Lewis,[37] in which lesions of the branches of the bundle rather than in the main stem have been demonstrated. In such cases, propagation of the impulse over the normal pathway is obviously impossible and some abnormality in stimulus production and stimulus distribution must occur. Electrocardiograms taken from these cases have shown the ventricular complexes to be characteristic of contractions arising in the right or left ventricle. It is with these clinical cases that experiments like those illustrated in figure 11 must be compared. If it is assumed that inhibitory fibers, derived from the vagus nerves, are distributed to the auriculoventricular bundle, they are probably distributed to its branches. The work of Engel (46), following that of Wilson (47), showing that nerve fibers are present in the branches makes this assumption more probable. Then if stimulation of the left vagus nerve produces an effect on the main stem of the bundle, it follows that the effect is probably continued downward along its branches. If this view of the distribution and function of the vagus nerves is correct, stimulation of the left nerve may depress the conductivity of the muscle of the main stem and branches of the A-V system to a degree so great that the ventricular muscle will receive no impulses conveyed by them. The ventricular muscle is then reduced to a condition directly comparable with that in those cases in which structural lesions in the branches of the A-V system have been demonstrated and in which the ventricular muscle was obliged to originate stimuli, if it originate them at all, in an atypical fashion and to distribute the stimuli over unusual paths yielding electrical curves of atypical form. It is probable that the mechanism suggested explains the occurrence of the atypical form of the curves in this group of experiments.

Kahn (48) had previously demonstrated the fact[38] "that automatic ventricular

[37] This case is to be published in a forthcoming number of *Heart*. References to electrocardiograms of this patient can be found in Lewis, Thomas, Mechanism of the Heart-Beat, London, 1911, p. 237.

[38] Kahn (48), *loc. cit.*, p. 633.

contractions which develop during artificial stimulation of the vagus are accompanied by atypical electrical curves." He states that "auriculoventricular dissociation in man, or section of the A-V bundle or its branches in the dog have shown in the investigations which have been made up to the present time that automatic ventricular contractions are expressed in the form of typical electrocardiograms. In dogs, on the contrary, dissociation caused by stimulation of the vagus nerve is accompanied by atypical electrocardiograms, while in cases of poisoning, both atypical and typical forms are found." He did not realize, however, that the phenomenon he was describing occurred only with stimulation of the left vagus. Kraus and Nicolai (49) and also Rothberger and Winterberg (11) have reported observations like those of Kahn. The latter have explained the ectopic ventricular contractions during vagus stimulation as due to a mechanical cause, such as the filling of the ventricular cavities.

It has been stated that when stimulation of the left vagus nerve causes ventricular asystole, presumably by depressing the power of the A-V system to conduct impulses, an independent rhythm will be initiated, depending upon the degree of irritability of some portion of the ventricular muscle. The degree of irritability may be so great that a ventricular rhythm is established practically immediately or within a very short time. On the other hand, the degree of irritability may be so low that the development of an idioventricular rhythm may be delayed until the stoppage has continued for some time. The occurrence of stoppage with the gradual development of an idioventricular rhythm has been studied in connection with the subject of experimental heart-block. Figures 10, A and B, 11, and 12 show degrees of irritability of the ventricular muscle measured by the length of time required to establish an independent ventricular rhythm. In figure 10, A and B, an independent ventricular contraction did not occur until 8.3 seconds after the beginning; that is to say, 0.6 of a second after the cessation of stimulation. None followed after that for about 2.3 seconds, the second one being in response to an auricular contraction, the depression of conductivity in the meanwhile having been relieved. In figure 11 there was practically no stoppage. In figure 12, experiment 644, an intermediate condition is seen. Stoppage of ventricular contractions lasted 2.4 seconds and the second beat followed the first after an interval of three seconds. These were both ectopic beats and they arose in the wall of the right ventricle. Ectopic beats may also arise in the left as well as in the right ventricle; in another curve taken during this experiment there were, in fact, three idioventricular contractions which arose in the left ventricle.

A combination of the effects described was sometimes seen, as in experiment 661 (figure 13, A and B). Stimulation of the left vagus arrested the contractions of both auricles and ventricles. Independent ventricular contractions were established before the auricular muscle escaped from the first effects of stimulation. The efficiency of the stimulus rapidly decreased and sequential contractions occurred, at a reduced rate but with a prolonged P-R (0.1 to 0.14–0.17) interval. In this dog, two varieties of effect due to stimulating the left vagus nerve were obtained. In other dogs, successive stimulations of this nerve resulted in effects first of one kind and later of another. In experiment 666, the first stimulation of the left vagus produced merely an increase in the length of the P-R interval. A second stimulation caused the heart to beat in a two to one rhythm, while a third

stimulation produced quiescence of the ventricular contractions, during which the auricular muscle continued to contract. Between the first and second stimulations nine seconds elapsed and between the second and third, six seconds. Seven other experiments (table I) occurred in this series in which more than one of the varieties of effects resulting from left vagus stimulation were obtained. In these cases the maximum effect was not always obtained the first time the nerve was stimulated.

In two experiments (Nos. 633 and 649) the reverse of the effects usually obtained were seen. In experiment 633, right vagus stimulation caused nomotopic ventricular contractions to cease, while the auricles continued to beat; a few ectopic ventricular contractions occurred. The left vagus in this case stopped the whole heart. In experiment 649, stimulation of the right vagus nerve produced a two to one block, while left vagus stimulation produced the effect usually brought about by stimulating the right nerve.

A single instance, experiment 662, was found in which stimulation of the left vagus nerve produced long periods of rest between groups of cardiac cycles, between successive single cycles, and between a single cycle and a group of cycles (figure 14, A and B). Each period of rest occupied a length of time that was approximately a multiple of the time occupied by a single cycle. The average duration of the single cycles during stimulation was 0.5 to 0.54 of a second; the periods of rest occupied 1.96, 1.6, 1.05, 1.6, and 1.57 seconds, each approximately four, three, or two times as long as the duration of the single cycles. Later in the curve the single cycles occupied 0.57 to 0.68 of a second, and the periods of rest, 0.99 to 1.67; these were again approximately multiples of the former. The fact that the periods of rest appear as multiples of the single cycles renders the interpretation probable that an incomplete block took place between the pace-maker and the rest of the heart. This experiment affords an example of sino-auricular block in the dog's heart. There was also lengthening of the P-R interval (0.1 to 0.17) during stimulation, the longest interval occurring in the first cycles of each group. The single cycles and the first cycles in each group are represented by complexes altered not only in size but in a minor degree also in form. Sino-auricular block has been obtained by Garrey (2)[39] by stimulating the vagus nerve in the turtle's heart, but in his case the phenomenon is described as resulting from the stimulation of the right vagus nerve by weak currents. He considered the block as a chronotropic effect, although he says inotropic and other effects were also present. In the present experiment the effect was obtained with the left vagus nerve, although four seconds after cessation of stimulation of the right vagus, a single cycle dropped out, and a quiescence equal to about the length of two took its place. There was no lengthening of the conduction time in the cycle after the pause.

DISCUSSION.

The description of the curves leads naturally to the conclusion that in dogs an essential difference exists between the right and left vagus nerves in their action on the heart. The difference is not merely a difference in degree, it is a more fundamental differ-

[39] Garrey (2), *loc. cit.*, p. 342.

TABLE I.

Experiment No.	Left vagus.[40]							Right vagus.[41]			
	1	2	3 A	3 B	4	0	Inv.	1	2	3	4
618		+						+			
619		+							+		
620	+	+								+	
621					+			+			+
623		+							+		
625				+					+		
626				+				+			
627		+						+			
628		+						+			
630						+			+		
632		+						+			
633				+			+	+			
634						+				+	
635		+		+				+			
636			+					+			
637		+							+		
638		+		+				+			+
639				+				+			+
640		+						+			
641		+						+			+
642		+						+			
643						+		+			
644				+				+			
645			+					+			+
646		+						+			
647						+		+			+
648				+				+			+

Experiment No.	Left vagus.[40]							Right vagus.[41]			
	1	2	3 A	3 B	4	0	Inv.	1	2	3	4
649		+					+			+	
650			+						+		
651					+	+[43]		+			+
652			+			+[43]		+			+
653	+[42]							+			
654	+[42]							+			
655			+					+			
656			+					+			
657			+					+			
658			+					+			
659			+					+			+
660	+								+		
660*a*		+							+		
661								+			+
662	+		+					+			
663	+							+			
664	+							+			
665				+				+			+
666	+	+	+					+			
667		+[42]	+					+			
668		+						+			
670		+						+			
671		+						+			
672		+						+			
673			+					+			
674		+	+						+		
675		+	+					+			

[40] Left vagus. Column 1. Experiments in which P-R time was lengthened. 2. Experiments in which incomplete block occurred. 3, A. Experiments in which ventricular asystole occurred. 3, B. Experiments in which ectopic ventricular systoles occurred. 4. Experiments in which both auricular and ventricular beats were slowed. 0. Experiments in which both nerves produced similar effects. Inv. = experiments in which inverted effects were produced by the two nerves.

[41] Right vagus. Column 1. Experiments in which the whole heart stopped. 2. Experiments in which the whole heart first stopped, but later beat at a slowed rate. 3. Experiments in which the whole heart was slowed. 4. Experiments in which the auricular beats were stopped and ectopic ventricular beats continued.

[42] After cessation of stimulation.

[43] During the period of stimulation.

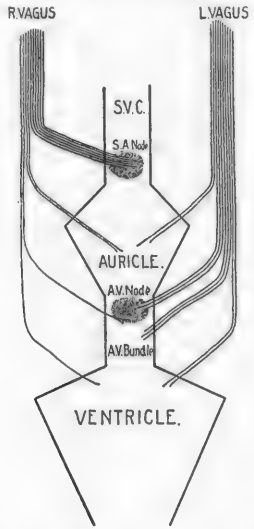
ence. Except in the two cases in which inversion of the usual result was obtained, auricular contractions were always arrested by successful stimulation of the right nerve while stimulation of the left nerve did not stop them. Nor did it ever happen on stimulating the right nerve, even when the rate was only slowed, that the depression of conduction from auricles was so great as to result in the incomplete or complete block of impulses derived from auricular contractions. On the other hand, such phenomena invariably resulted from left vagus stimulation. Other experimenters, already mentioned, have described auricular slowing with ventricular quiescence during vagus stimulation. But as far as the writer is aware it has not been recognized that this combination of phenomena is usually produced by stimulation of the left vagus and not by stimulation of the right. As recently as 1911, Dogiel (36) stated[44] that "many physiologists have called attention to the fact that stimulation of the right vagus has not the same effect on the heart action as the left, a result due in part to the strength and duration of the stimulus"; and von Tabora (50), working in Hering's laboratory on dogs, said that "vagus action alone may possibly on occasions produce ventricular silences, but cannot bring about dissociation."

It is probable that the difference in function between the two vagi is dependent upon the development of the heart and its conducting mechanism, and of the nerves in relation to the muscular structures. An explanation of the differences must therefore await a further development of knowledge regarding these embryological points. The existence of a homolateral distribution of the two vagus nerves in the hearts of turtles has been indicated by Mills (21) and has been proved by Garrey (2). A similar roughly homolateral distribution of the vagi in dogs has also been described by Lim Boon Keng (51), and of the accelerator nerves by von Schumacher (52), but it is doubtful whether the distribution that has been described is as refined as is necessary to explain the results obtained in the experiments described in this paper.

In text-figure 2 an attempt has been made to indicate a possible distribution of the two nerves which might explain their differences

[44] Dogiel (36), *loc. cit.*, p. 122.

in function. Such a distribution, of course, is purely hypothetical and the diagram is offered simply for the purpose of rendering the experimental findings more comprehensible.



TEXT-FIG. 2. The superior vena cava (S.V.C.), the sino-auricular node, the mass of both auricles, the auriculoventricular node and bundle, and the mass of both ventricles are shown diagrammatically. The right vagus nerve is shown to send a preponderating number of fibers to the S.A. node, a smaller number to the auricles and to the A-V system and the ventricles. The distribution on the left side is reversed; none are sent to the S.A. node, and a larger number to the A-V node and A-V bundle and the ventricles.

To each vagus nerve are accredited an equal number of fibers. The greater number of those of the right nerve are represented as entering the sino-auricular node; they exercise, therefore, a preponderating chronotropic effect on the whole heart. A smaller number are distributed to the auricles and to the ventricles, where they exercise inotropic effects far greater on the auricle than on the ventricle. Of the fibers assigned to the left vagus nerve, none enter the sino-auricular node. It might have been more in consonance with the findings in these experiments, however, to have shown a fiber entering the sinus node. Fibers are shown to enter

the auricles where they have an inotropic effect, while others enter the ventricles where they have an inotropic and possibly a bathmotropic action. Most of the fibers are shown to enter the auriculoventricular system, for the production of a dromotropic effect is the most conspicuous function of this nerve. It must be added that the diagram, as an explanation even of this paper, does not elucidate all the phenomena found, and is included with hesitancy, lest it be supposed to contain a definitive opinion.

CONCLUSIONS.

It may be concluded from the results obtained in these experiments:

1. That stimulation of the right vagus nerve in the dog usually causes arrest of all the chambers of the heart.

2. That stimulation of the left vagus nerve exerts a moderate negative chronotropic effect on the auricles.

3. That stimulation of the left vagus nerve has a profound effect on the conduction of impulses over the auriculoventricular system.

4. That the degree of effect exercised on the auriculoventricular system by stimulation of the left vagus nerve varies. In some dogs conduction is depressed to an extent which causes only a delay in the conduction of impulses from auricles to ventricles (P-R time); in other dogs the conduction is depressed to a degree which results in incomplete heart-block; while in still other dogs conduction is so depressed that although the auricles continue to contract, no impulses pass from them to the ventricles.

5. That when stimulation of either the right or left vagus nerve causes asystole of nomotopic ventricular contractions, ectopic ventricular contractions may occur.

6. That the time which elapses before ectopic ventricular contractions occur depends upon the irritability of the ventricular muscle, and this may vary in different dogs.

7. That stimulation of the left vagus nerve may rarely cause sino-auricular block. Possibly stimulation of the right nerve may also produce this effect.

8. That there is consequently usually a great qualitative difference in the action of the two vagus nerves on the heart of the dog.

BIBLIOGRAPHY.

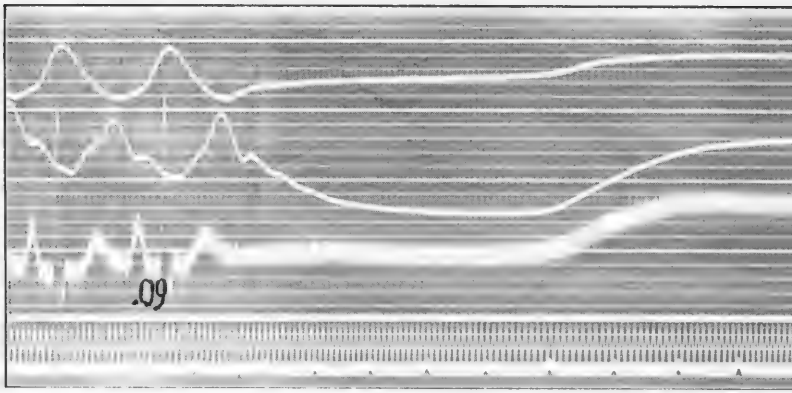
1. Meyer, A. B., Das Hemmungsnervensystem des Herzens, Berlin, 1869, 1.
2. Garrey, W. E., *Am. Jour. Physiol.*, 1911, xxviii, 330.
3. Robinson, G. C., and Draper, G., *Jour. Exper. Med.*, 1911, xiv, 217; 1912, xv, 14.
4. Cohn, A. E., *Jour. Exper. Med.*, 1912, xv, 49.
5. McWilliam, J. A., *Jour. Physiol.*, 1888, ix, 167.
6. François-Franck, *Arch. de physiol. norm. et path.*, 1890, Series 5, ii, 395.
7. Roy, C. S., and Adami, J. G., *Phil. Tr. Roy. Soc. London*, 1893, clxxxiii, 199.
8. Bayliss, W. M., and Starling, E. H., *Jour. Physiol.*, 1892, xiii, 407.
9. Rehfisch, E., *Arch. f. Physiol.*, 1906, Suppl., 152.
10. Hering, H. E., *Arch. f. d. ges. Physiol.*, 1909, cxxvii, 155.
11. Rothberger, C. J., and Winterberg, H., *Arch. f. d. ges. Physiol.*, 1911, cxli, 343.
12. Rothberger, C. J., and Winterberg, H., *Arch. f. d. ges. Physiol.*, 1910, cxxxv, 506.
13. McWilliam, J. A., *Jour. Physiol.*, 1885, vi, 192.
14. Gaskell, W. H., *Phil. Tr. Roy. Soc. London*, 1883, clxxiii, 993.
15. Hofmann, F., *Arch. f. d. ges. Physiol.*, 1895, lx, 139.
16. Guyénot, E., *Compt. rend. Soc. de biol.*, 1907, i, 1145.
17. McWilliam, J. A., *Jour. Physiol.*, 1885, vi, p. xvi.
18. Gaskell, W. H., *Jour. Physiol.*, 1882, iii, 369.
19. Gaskell, W. H., *Jour. Physiol.*, 1883, iv, 43.
20. Mills, T. W., *Jour. Physiol.*, 1884, v, 359.
21. Mills, T. W., *Jour. Physiol.*, 1885, vi, 246.
22. Kazem-Beck, *Arch. f. Anat. u. Physiol.*, 1888, 325.
23. Kronecker, H., *Arch. internat. de physiol.*, 1904–5, ii, 211.
24. Guyénot, E., *Compt. rend. Soc. de biol.*, 1907, i, 1025.
25. Guyénot, E., *Compt. rend. Soc. de biol.*, 1907, i, 1032.
26. Mills, T. W., *Jour. Anat. and Physiol.*, 1886–7, xxi, 1.
27. Mills, T. W., *Jour. Anat. and Physiol.*, 1887–8, xxii, 1.
28. Gaskell, W. H., *Jour. Physiol.*, 1885, vii, 1.
29. Jürgens, H., *Arch. f. d. ges. Physiol.*, 1909, cxxix, 506.
30. Stübel, H., *Arch. f. d. ges. Physiol.*, 1910, cxxxv, 249.
31. Arloing, S., and Tripier, L., *Arch. de physiol. norm. et path.*, 1871, iv, 411, 588.
32. Knoll, P., *Sitzungsb. d. k. Akad. d. Wissensch., Math.-Naturwiss. Kl., Abt. III*, 1872, lxvi, 195.
33. Pawlow, J. P., *Arch. f. Physiol.*, 1887, 498.
34. Einthoven, W., *Arch. internat. de physiol.*, 1906–7, iv, 132.
35. Einthoven, W., *Arch. f. d. ges. Physiol.*, 1908, cxxii, 517.
36. Dogiel, J., *Arch. f. d. ges. Physiol.*, 1911, cxlii, 109.
37. Arloing, S., and Tripier, L., *Arch. de physiol. norm. et path.*, 1873, v, 157.
38. Legros, C., and Onimus, *Jour. de l'anat. et de la physiol.*, 1872, viii, 561.
39. Hunt, R., and Harrington, D. W., *Jour. Exper. Med.*, 1897, ii, 723.
40. Masoin, E., *Bull. de l'Acad. roy. de méd. de Belg.*, 1872, vi, 388.
41. Langendorff, O., *Mitt. a. d. Königsberger physiol. Laboratorium*, 1898, 68. Reviewed in *Centralbl. f. d. med. Wissensch.*, 1879, xvii, 406.
42. Johansson, J. E., and Tigerstedt, R., *Mitt. a. d. physiol. Laboratorium in Stockholm*, 1889, vi, No. 8. Reprinted in *Hygica*, Festband, 1889, No. 5, 1.

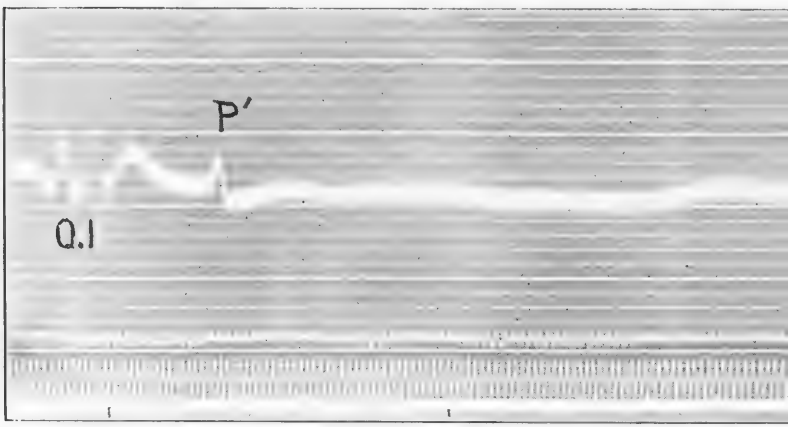
EXPLANATION OF PLATES.

PLATE 60.

FIG. 1. (Experiment 673.) Stimulation of the right vagus nerve. The curves from above downward are: (1) suspension curve of right auricle, (2) suspension curve of right ventricle, (3) electrocardiogram, (4) signal showing the length of stimulation, (5) time in 0.02 of a second, (6) time in 0.2 of a second. The stimulus applied stopped the whole heart. The P-R time was lengthened slightly, 0.09 to 0.12 of a second.

FIG. 2. (Experiment 666.) Stimulation of the right vagus nerve. No suspension curves are shown here or in the following curves. An auricular contraction (P′) occurs shortly after the beginning of stimulation. Three seconds later a complete cycle, and 2.8 seconds later a second cycle escapes; between them occurs one blocked auricular (P′) wave. The P-R time in the escaped cycles was lengthened 0.1 to 0.12 and 0.14 of a second.


.09


P'
0.1

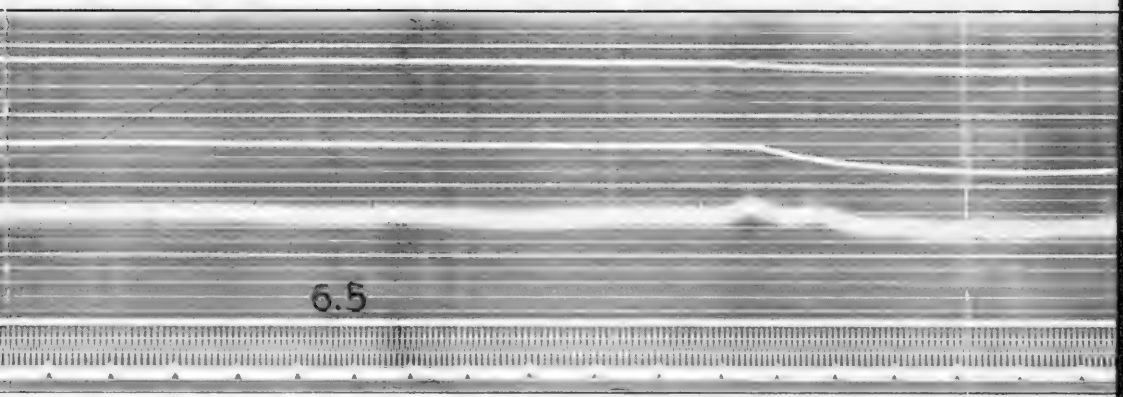


FIG. 1.

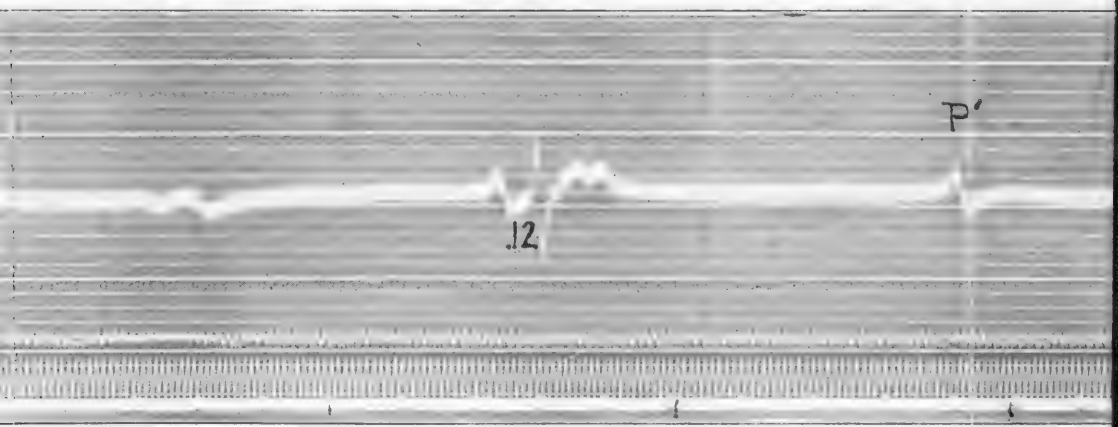
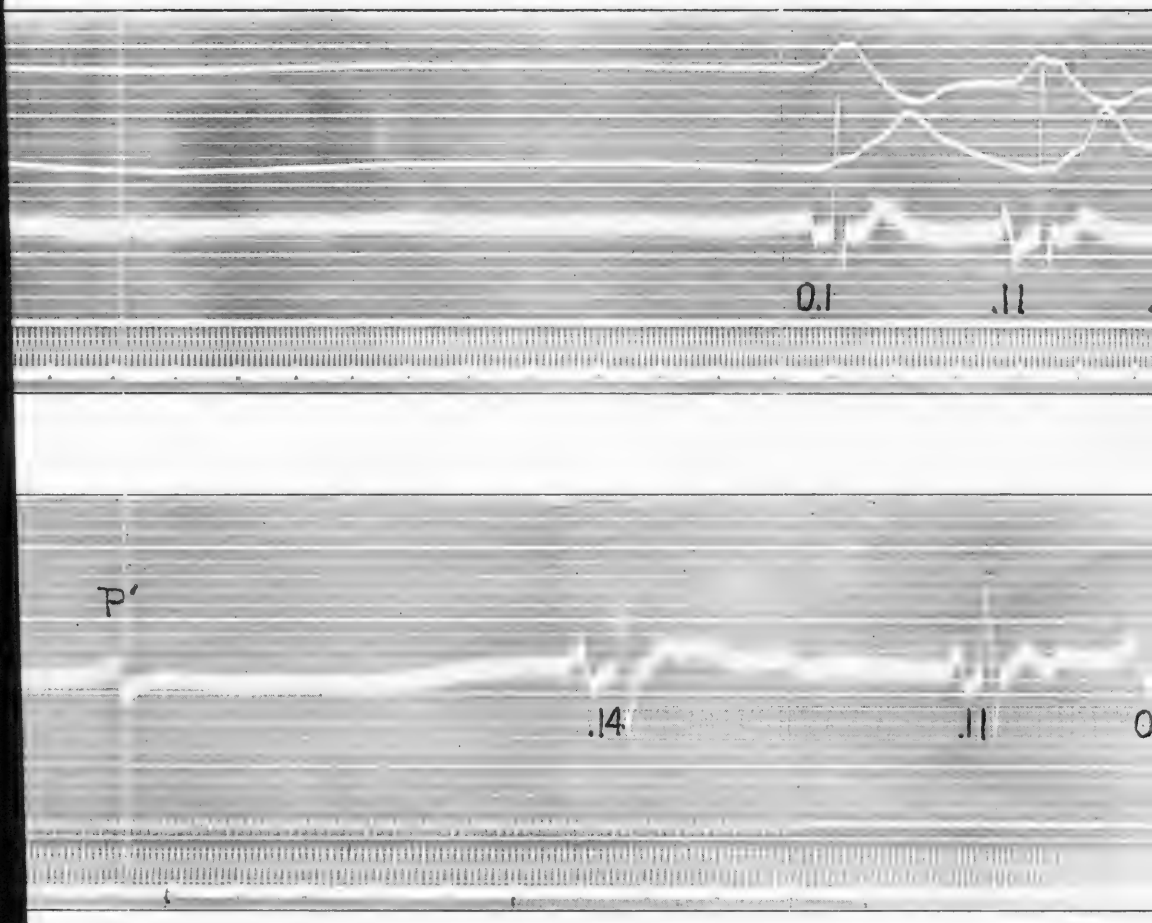


FIG. 2.


0.1
.11
P'
.14
.11

TE 69.

erves.

PLATE 70.

FIG. 3. (Experiment 666.) Stimulation of the right vagus nerve. After the cessation of stimulation the first cycle shows a lengthened P-R interval (0.1 to 0.12 of a second), after which a blocked auricular contraction occurs.

FIG. 4. (Experiment 659). Stimulation of the right vagus nerve. Shortly after the beginning of stimulation, one blocked auricular (P′) contraction occurs. During the period of stimulation two ectopic (right ventricular) contractions are seen. After stimulation the P-R interval is lengthened, 0.11 to 0.18 of a second, and two blocked auricular (P′) waves occur.

FIG. 5. (Experiment 661.) Stimulation of the right vagus nerve. The auricles cease contracting entirely. The complexes representing ventricular contractions are derived from stimuli arising in the wall of the left ventricle. The first two auricular contractions after the cessation of stimulation are blocked. The P-R time is lengthened from 0.11 to 0.14 of a second. It returned to normal in the third cycle after the last one reproduced.

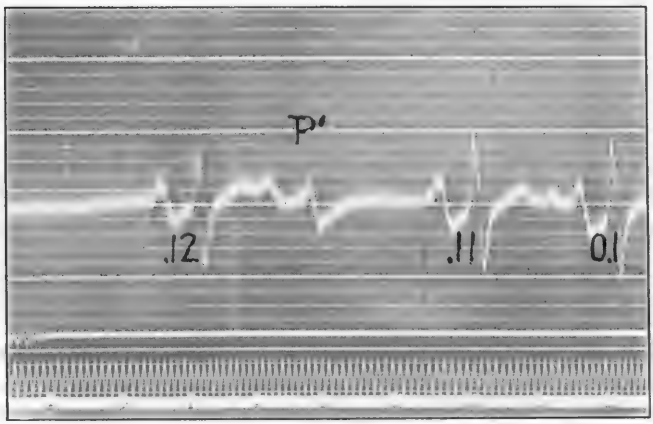
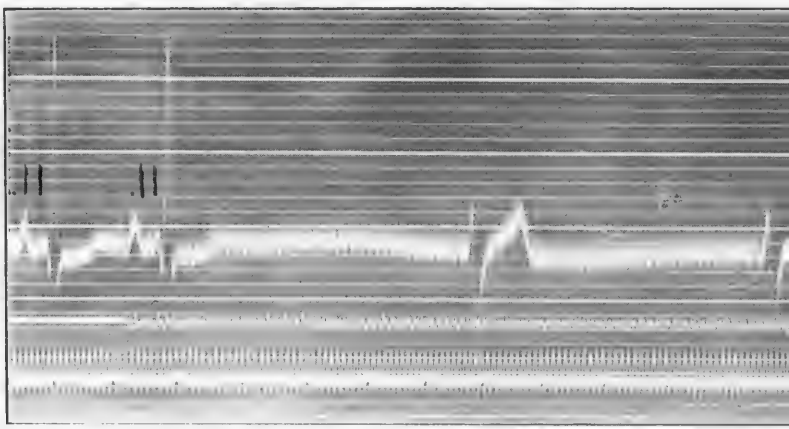


FIG. 3.



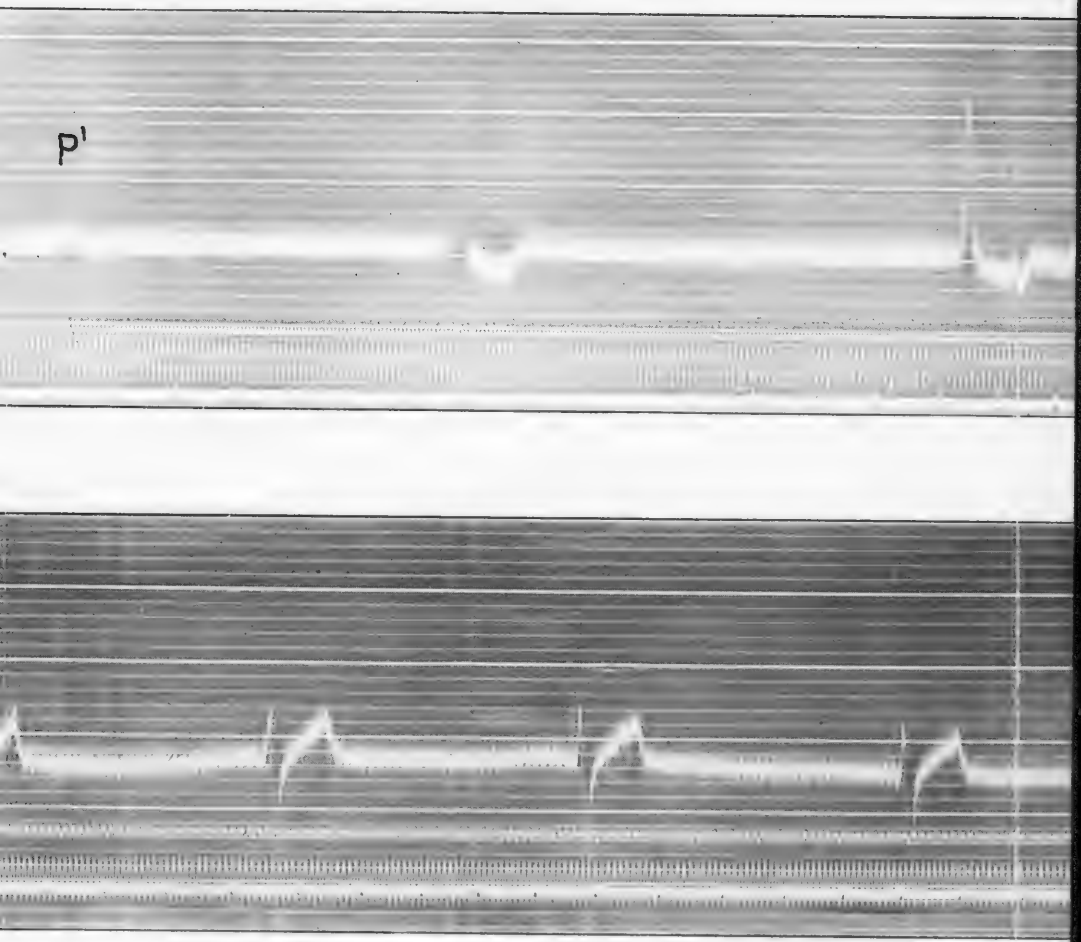


Fig. 5.

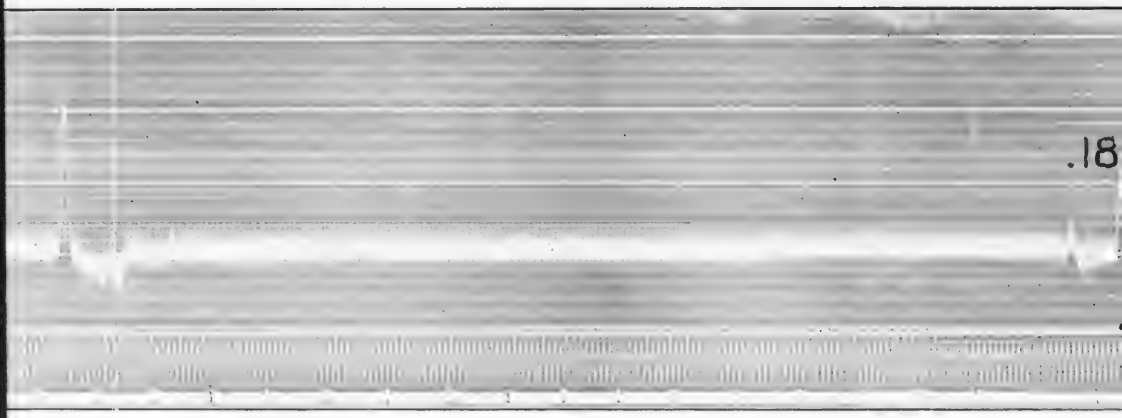
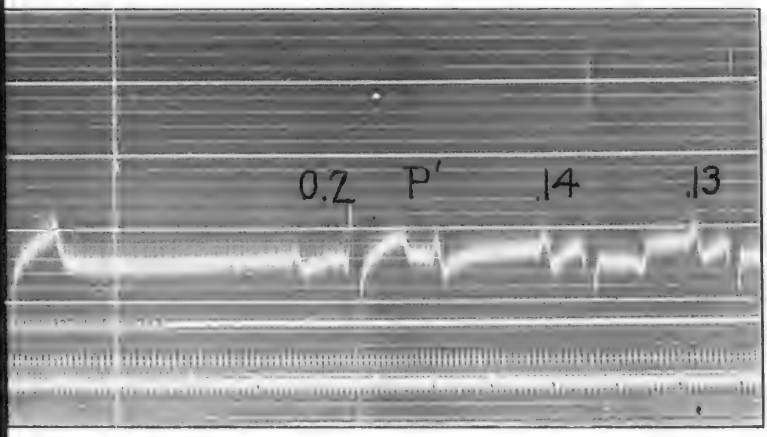


FIG. 4.



COHN: Effects of Stimulation of Two Vagus Nerves.

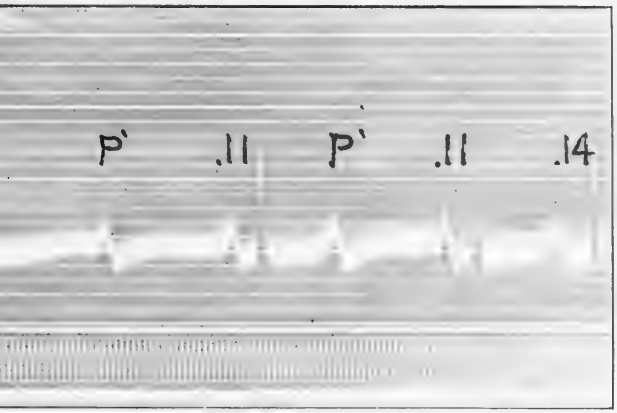

P'
.11
P'
.11
.14

PLATE 71.

FIG. 6.[45] (Experiment 639.) Stimulation of the right vagus nerve. Similar to the effect shown in figure 5, except that the ventricular complexes in this curve originate in the right ventricle. The first three auricular (P) waves after the end of stimulation are dissociated from the ventricular complexes.

FIG. 7. (Experiment 662.) Stimulation of the left vagus nerve. Gradual increase in the length of the P-R time, 0.1 to 0.24 of a second, is shown. The fifth cycle after the last one reproduced has a P-R interval of 0.12 of a second.

[45] In figure 6, on account of the faintness of the original tracings, the R waves of the ventricular complexes have been redrawn in the reproduction.

.08

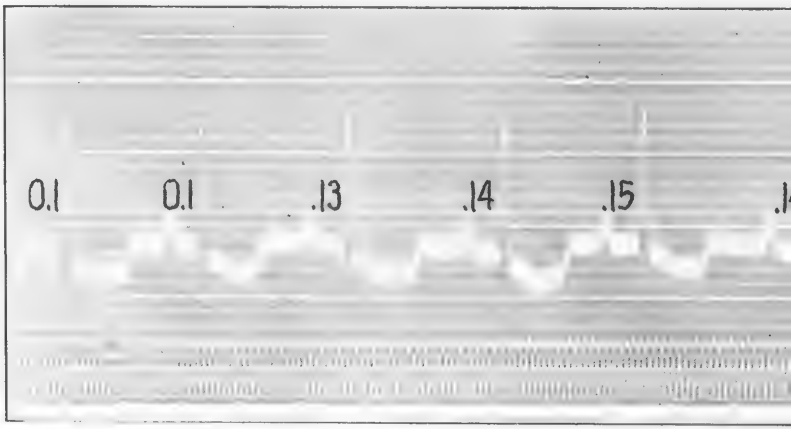

0.1
0.1
.13
.14
.15

Fig. 6.

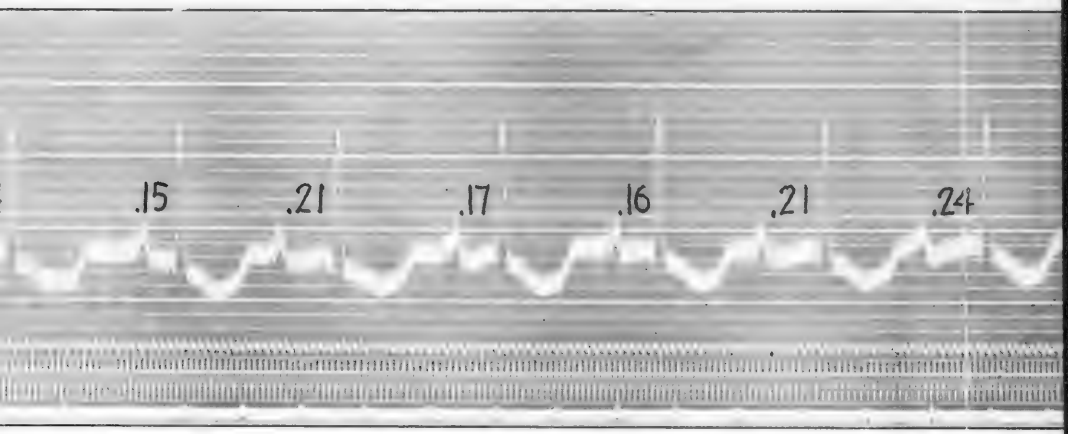
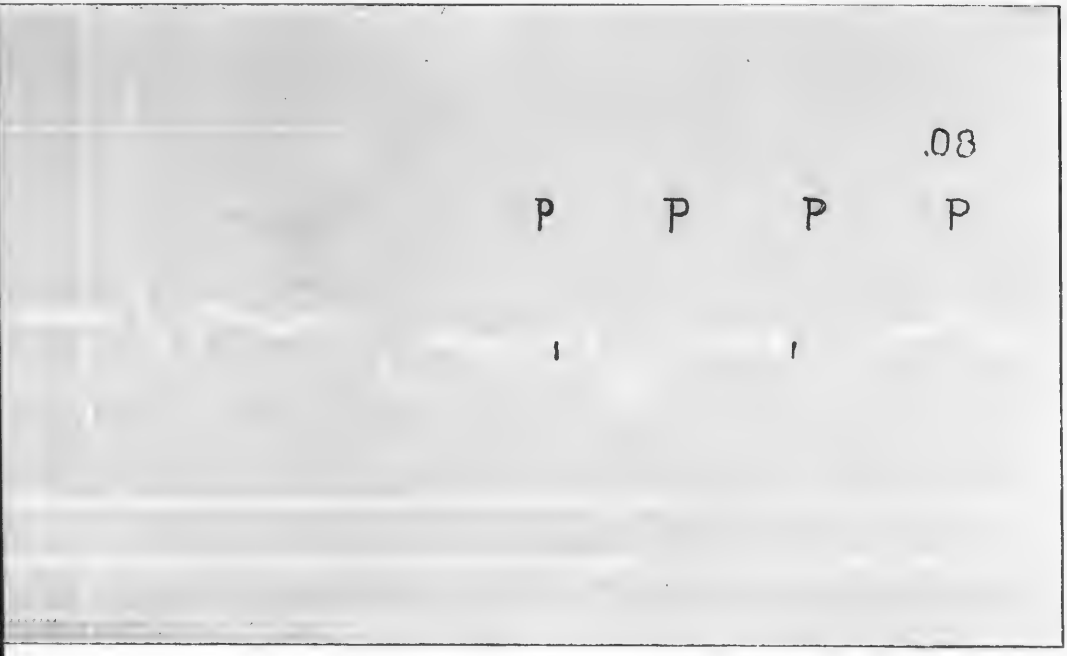
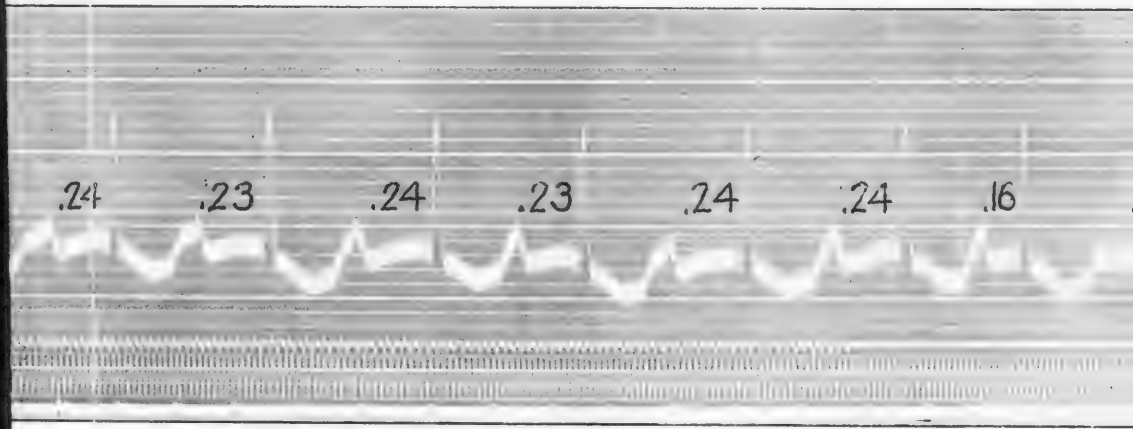
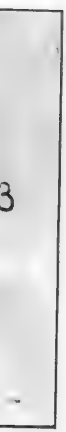


Fig. 7.


.08
P
P
P
P


.24
.23
.24
.23
.24
.24
.16

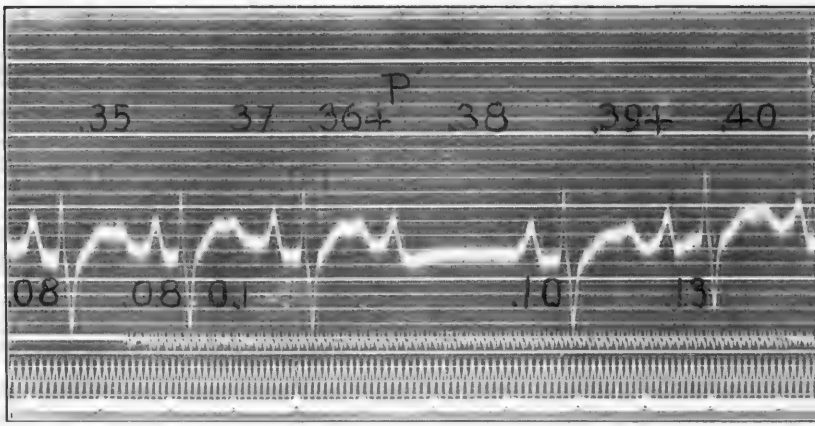


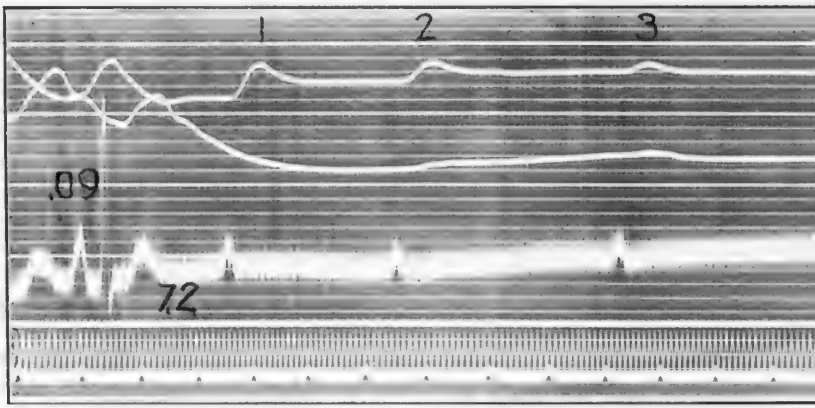
Nerves.

PLATE 72.

FIG. 8. A and B. (Experiment 666.) The second part, B, of this figure is directly continuous with the first portion. The decimals above the electrocardiogram represent the intervals (P-P) between succeeding auricular contractions; the decimals underneath, the P-R interval. The P-R interval in the groups of cycles in figure 8, A, gradually increases until an auricular wave is blocked (P′). In figure 8, B, the last cycle in the group shown has a P-R interval slightly shorter than the cycle immediately preceding.

FIG. 9. (Experiment 673.) Stimulation of the left vagus nerve. Suspension curves as in figure 1. The auricular contractions in the suspension curve gradually become smaller during the eight numbered beats until no elevations are seen on the curve. Ventricular contractions have ceased. The electrical curve shows the P waves, the representatives of auricular contractions, present. After stimulation, the P-R interval is lengthened from 0.09 to 0.12 of a second. An auricular contraction, P′, is then blocked and the P-R time lengthens to 0.14 of a second. The fourth cycle after the last one reproduced shows a P-R time of 0.11 of a second.


P
.35
37
36+
38
39+
40
08
08
0.1
.10
13


1
2
3
09
72

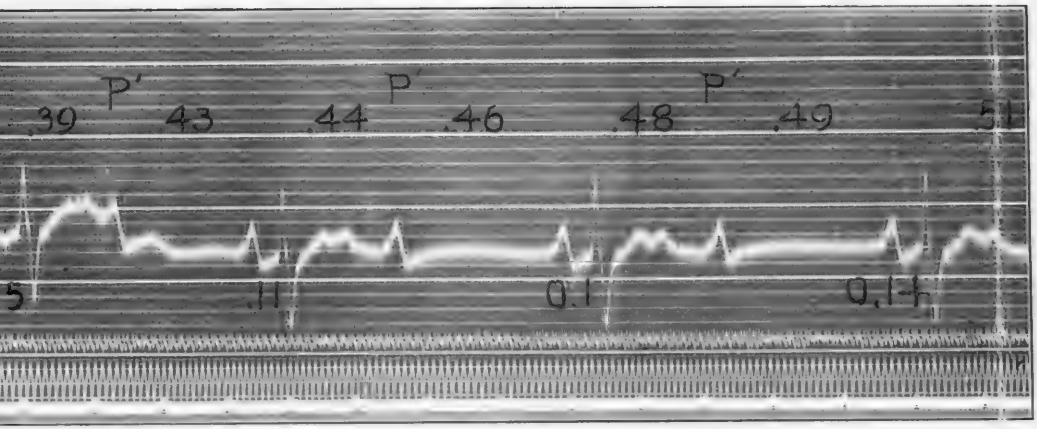


FIG. 8A.

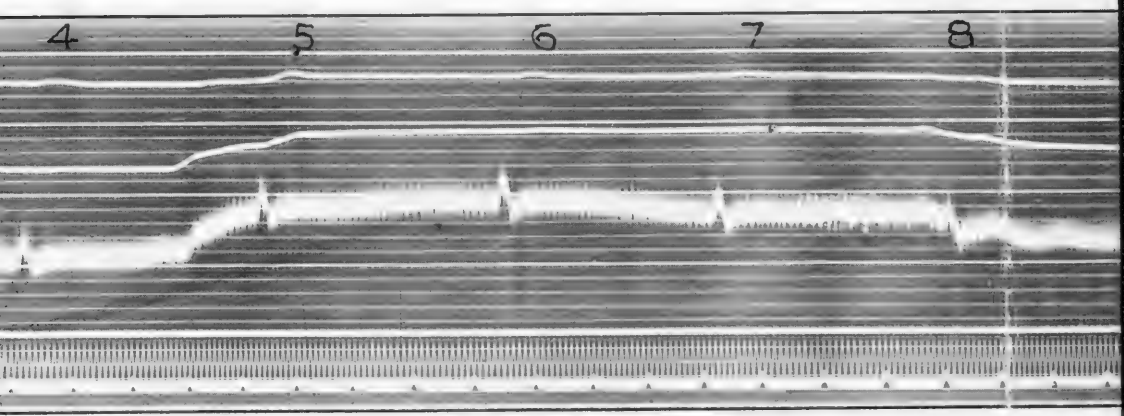


FIG. 9.

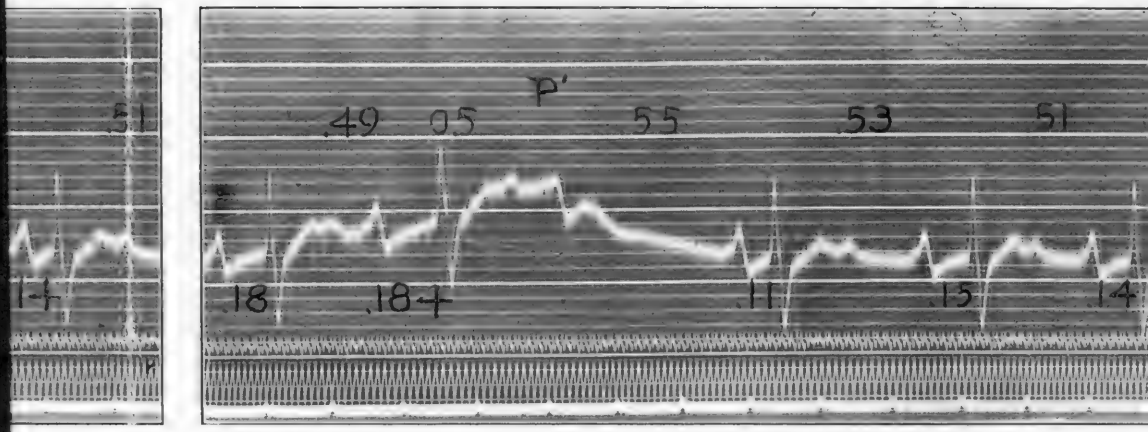
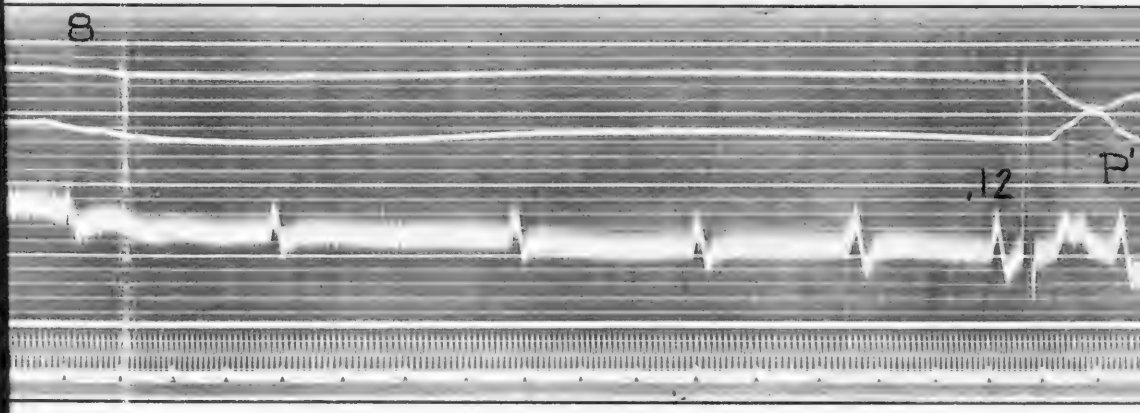
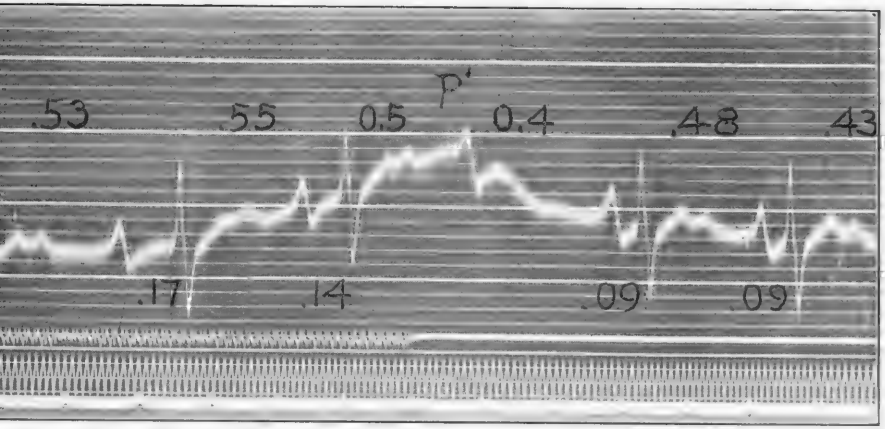
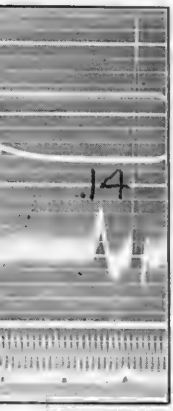


FIG. 8E



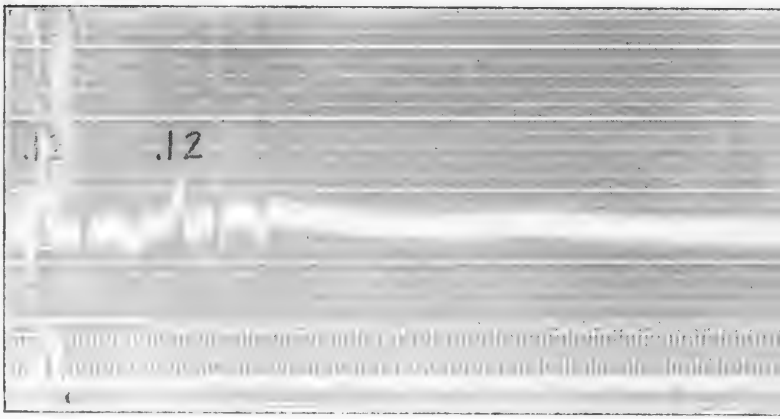


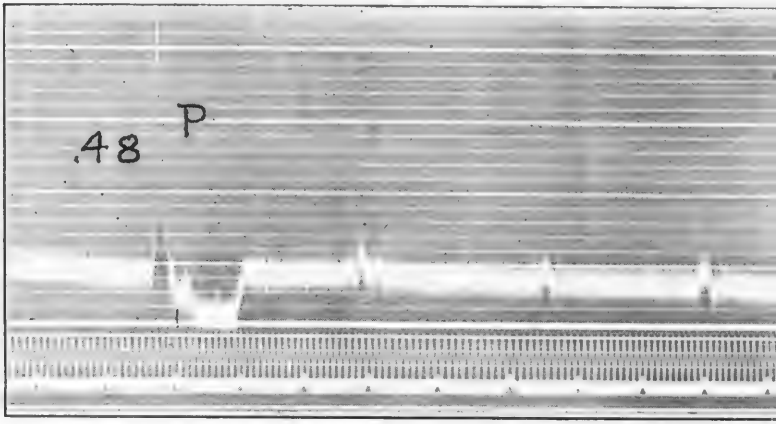


n of Two Vagus Nerves.

PLATE 73.

FIG. 10. A and B. (Experiment 667.) Stimulation of the left vagus nerve. The two curves are directly continuous. Figure 10, A, shows events like those of figure 9. It differs in that a pause of 2.3 seconds occurs after the beginning of stimulation and until the auricular contractions begin. Figure 10, B, shows an escaped ectopic (right ventricular) complex. The remainder of the curve shows a 2:1 rhythm. The rest of this curve, which is not reproduced, shows ten additional cycles of a 2:1 rhythm, a sequential cycle, another 2:1 cycle, and finally a sequential rhythm.


.12


.48
P

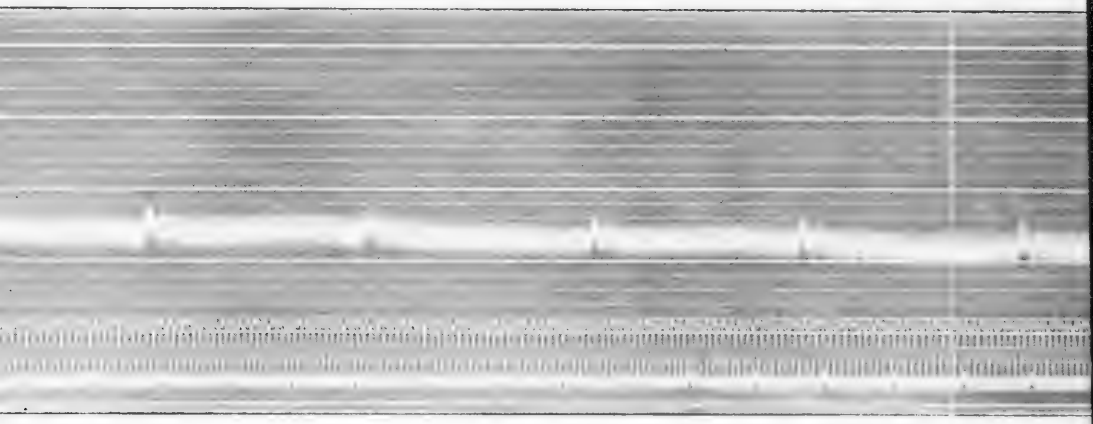


Fig. 10A.

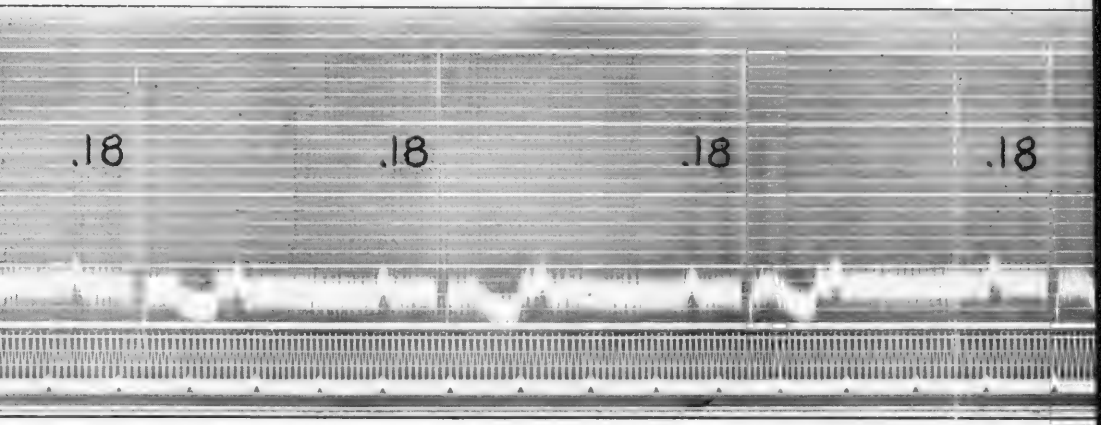
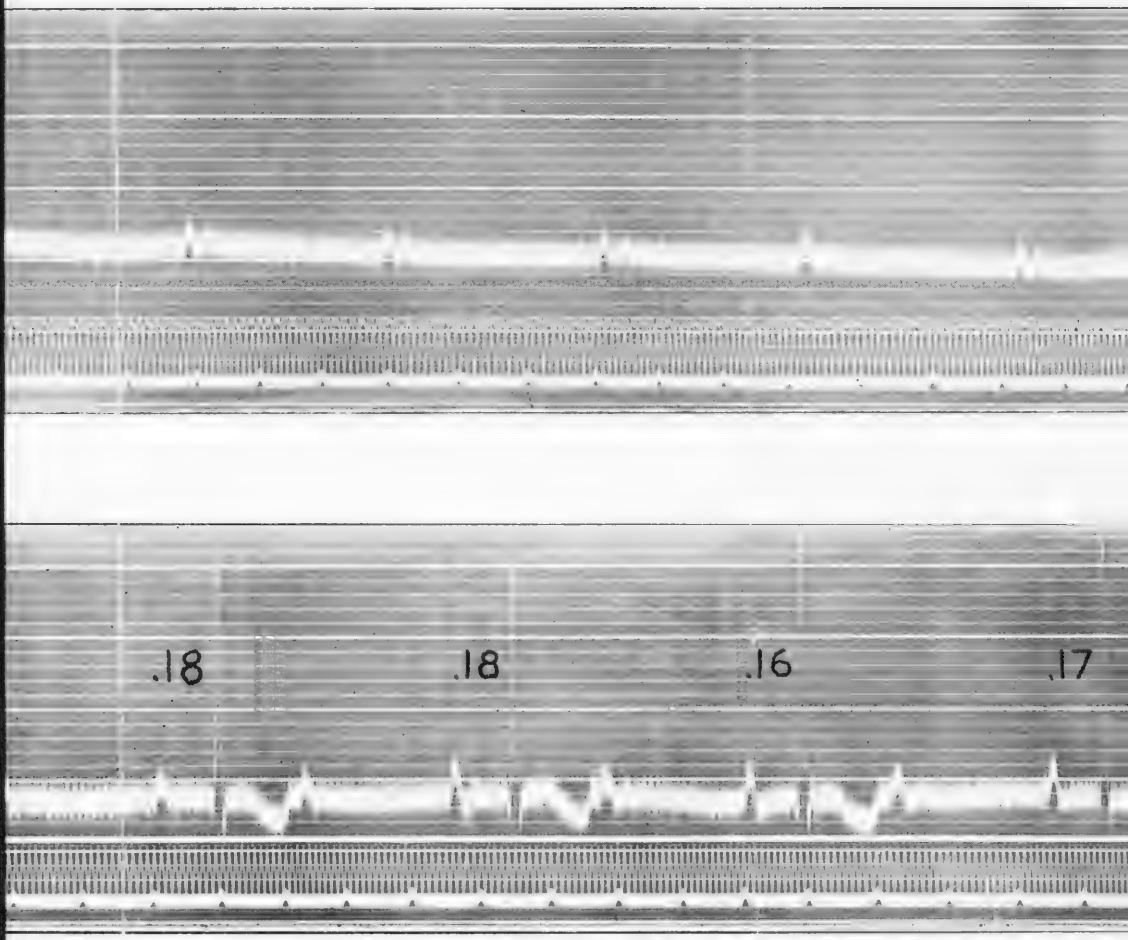


Fig. 10B.


.18
.18
.16
.17

LATE 73.

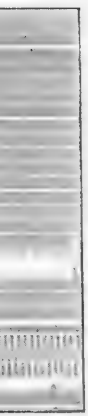
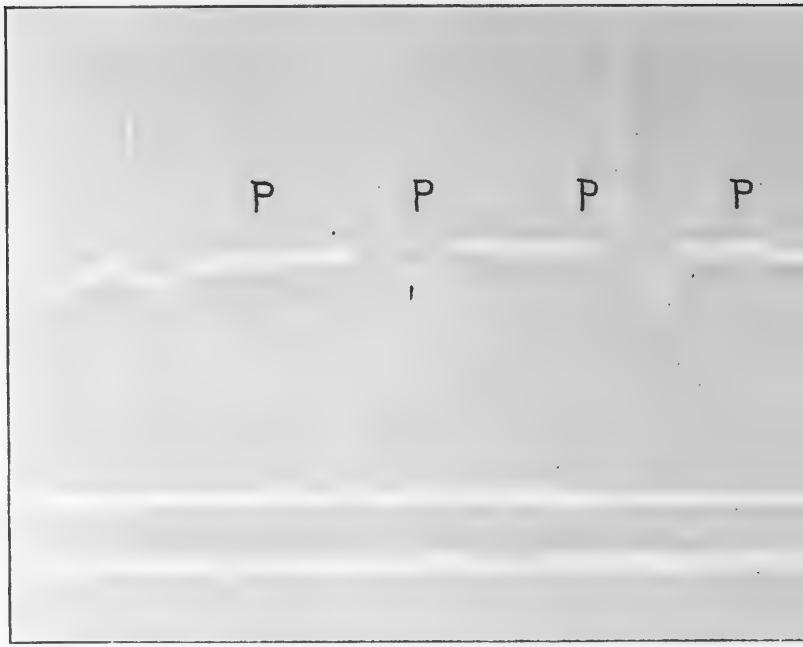


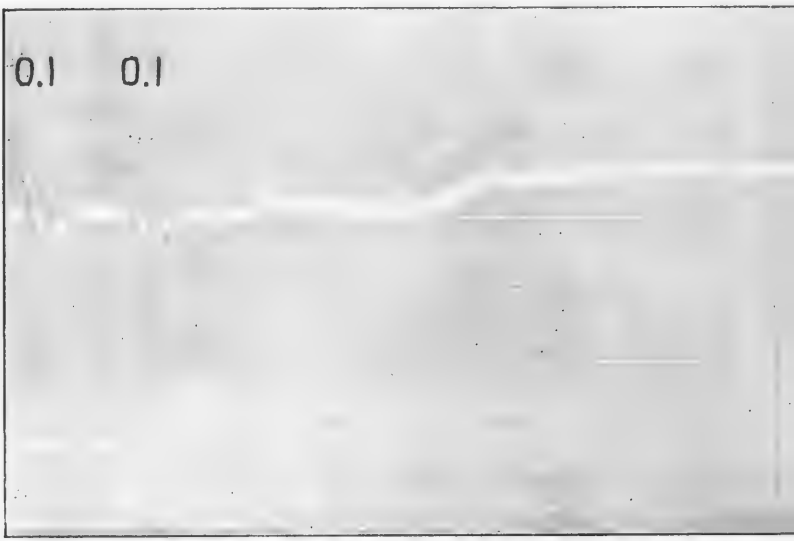
PLATE 74.

FIG. 11.[46] (Experiment 639.) Stimulation of the left vagus nerve. The auricular contractions continue. Nomotopic ventricular contractions have ceased. Their place is taken by ectopic ventricular contractions arising in the right ventricle. Auricular and ventricular contractions occur in complete dissociation.

FIG. 12. (Experiment 644.) Stimulation of the left vagus nerve. Auricular contractions continue throughout the period of stimulation. Two ventricular contractions originating in the wall of the right ventricle appear. The P-R time after stimulation is 0.18 of a second. One auricular contraction (P) is then blocked, after which the normal sequence is restored.

[46] In figure 11, on account of the faintness of the original tracings, the R waves of the ventricular complexes have been redrawn in the reproduction.


P
P
P
P


0.1
0.1

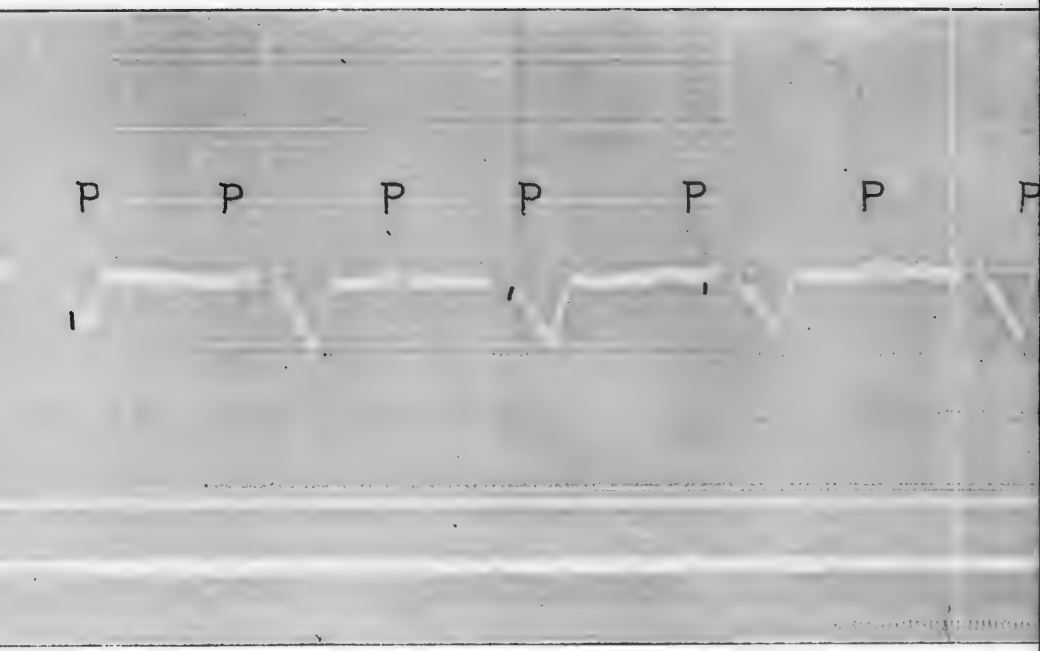


Fig. 11.

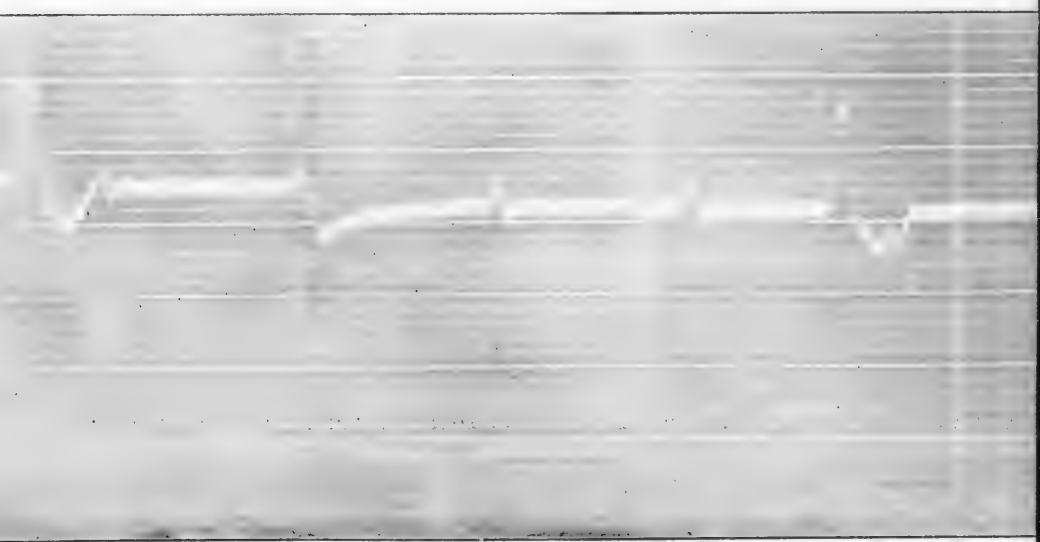
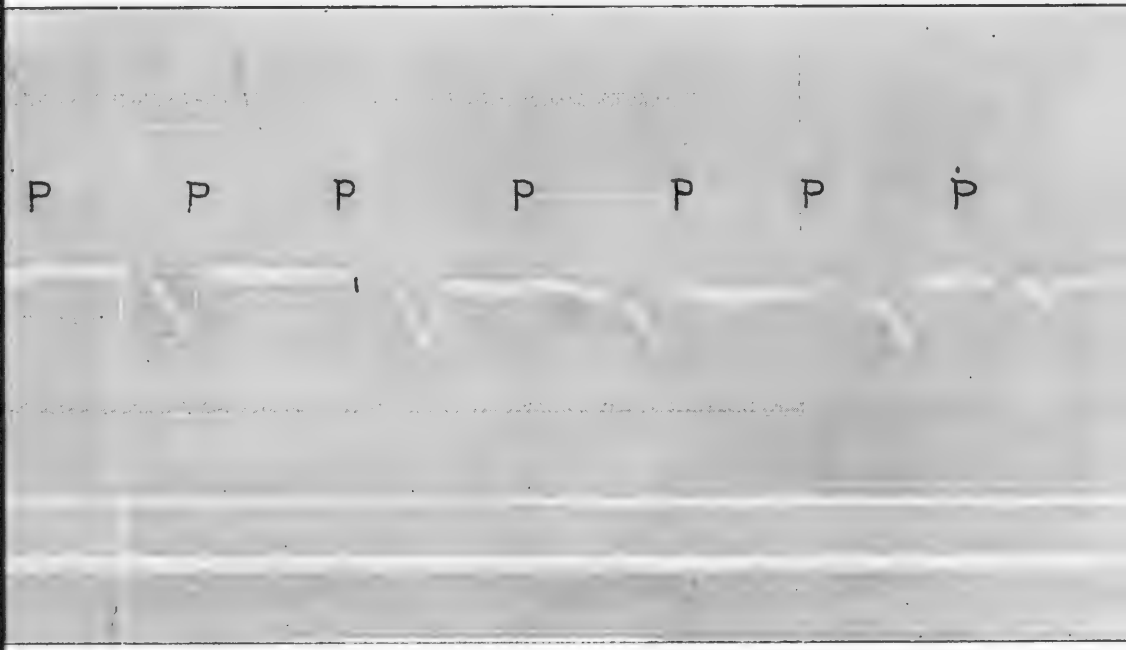
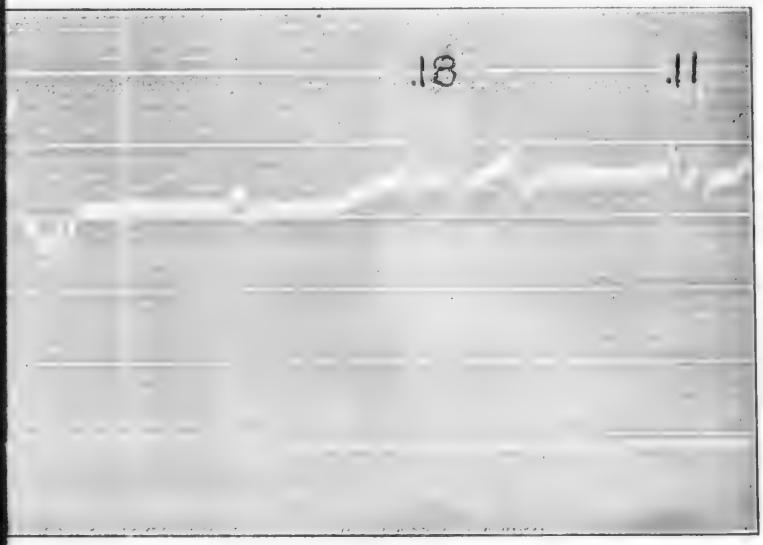


Fig. 12.

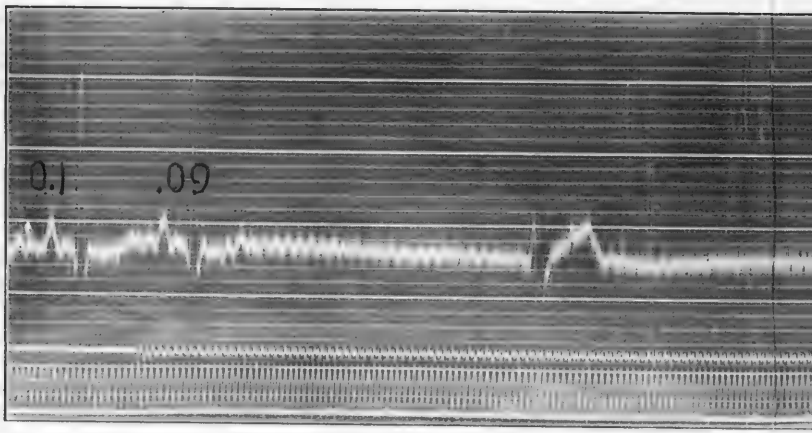


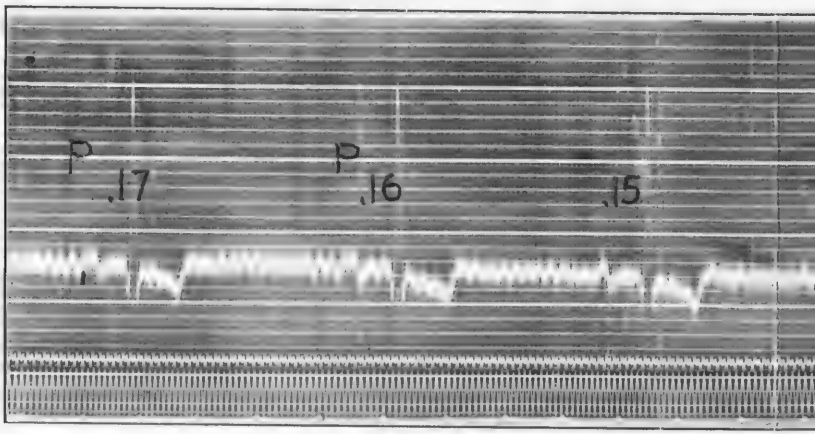


COHN: Effects of Stimulation of Two Vagus Nerves.

PLATE 75.

FIG. 13. A and B. (Experiment 661.) Stimulation of the left vagus nerve. B is directly continuous with A. In A auricular contractions first ceased. Four are seen toward the end of the curve. Four ventricular contractions are present. These are ectopic (left ventricular) systoles. The last cycle in A and all the cycles in B are sequential and nomotopic, but are slowed and show a prolonged P-R interval (0.1 to 0.17 of a second).


0.1
.09


P
.17
P
.16
.15

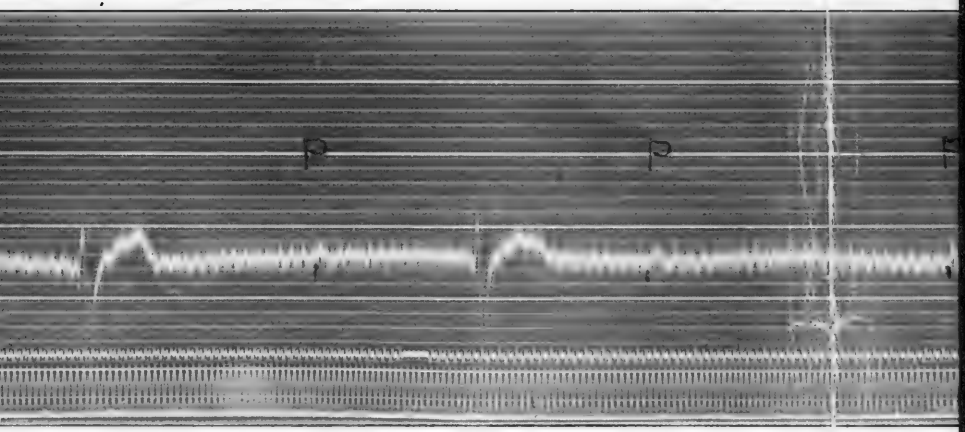


Fig. 13A.

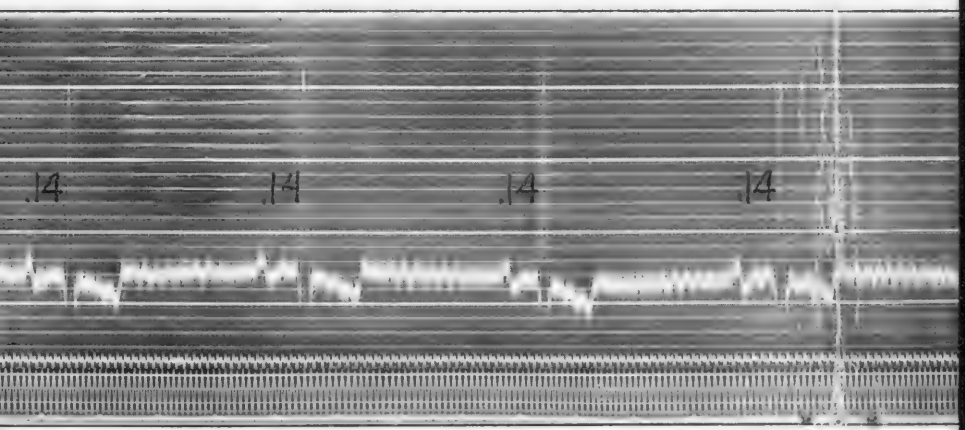
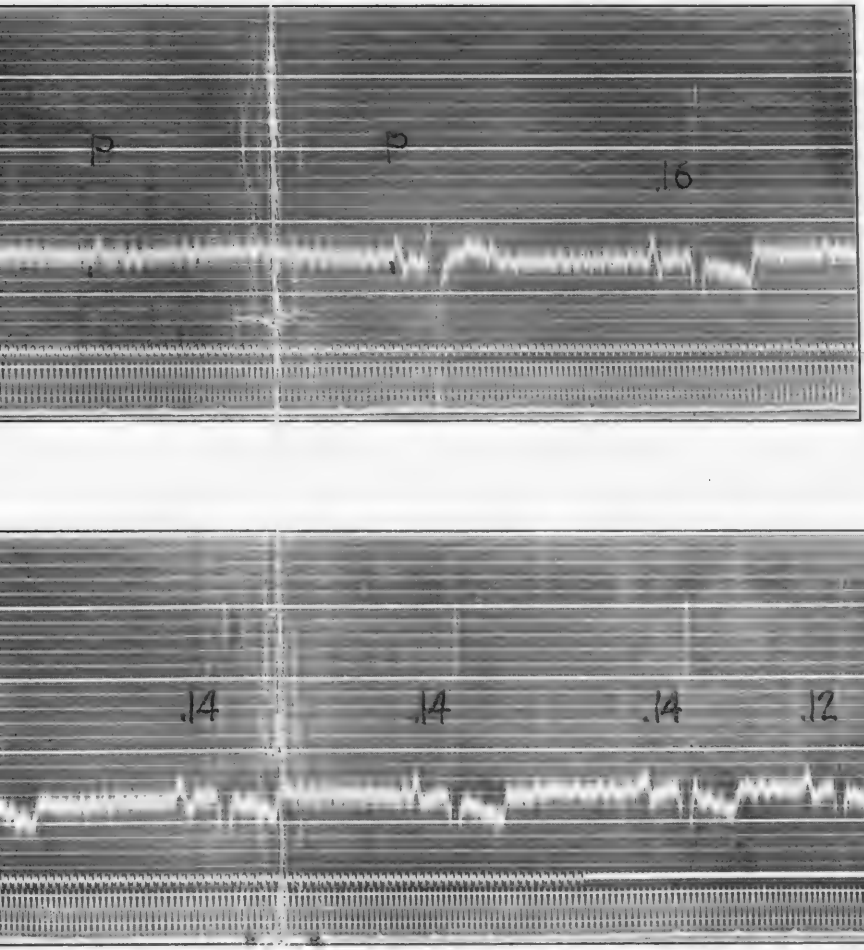


Fig. 13B.

Fig. 13A.

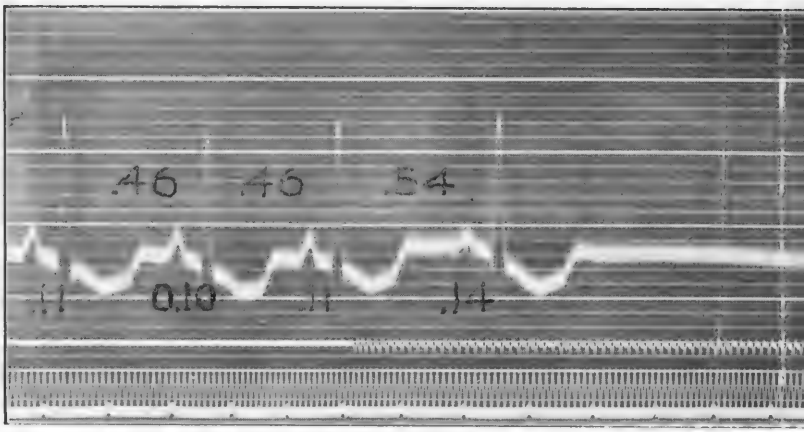
Fig. 13B.

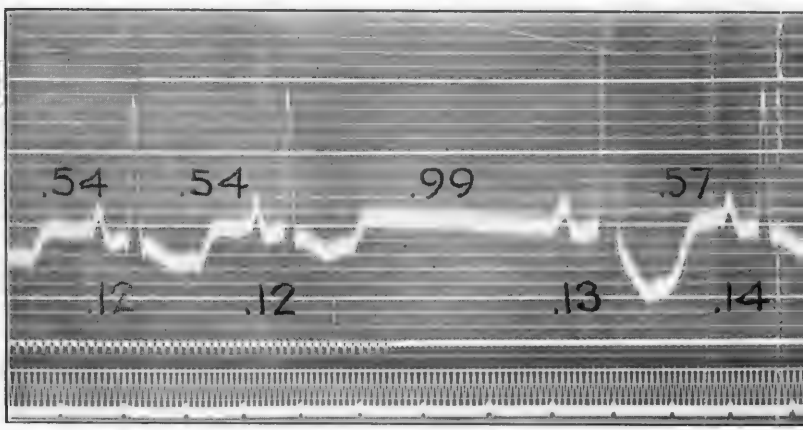


COHN: Effects of Stimulation of Two Vagus Nerves.

PLATE 76.

FIG. 14. A and B. (Experiment 662.) Stimulation of the left vagus nerve. The two portions of the curve are not directly continuous. Nine cycles are omitted. The decimals above the electrocardiogram represent the interauricular time; those below, the P-R time. The curve represents an instance of sino-auricular block. For the details the reader is referred to the text (p. 752).


.46
.46
.54
.11
0.10
.11
.14


.54
.54
.99
.57
.12
.12
.13
.14

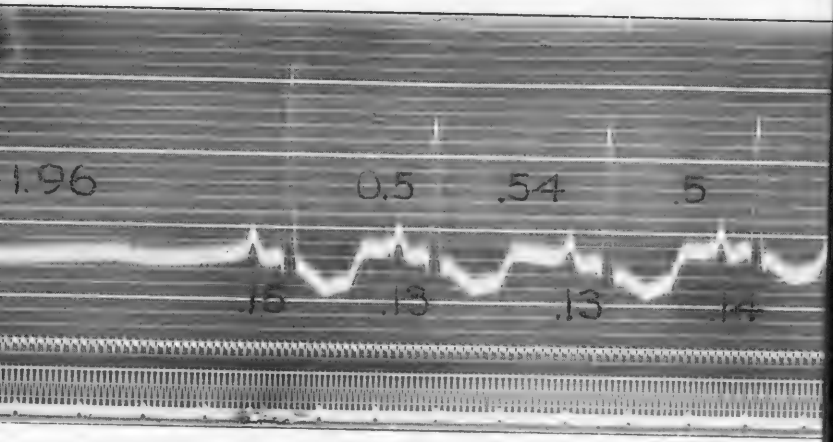


Fig. 14A.

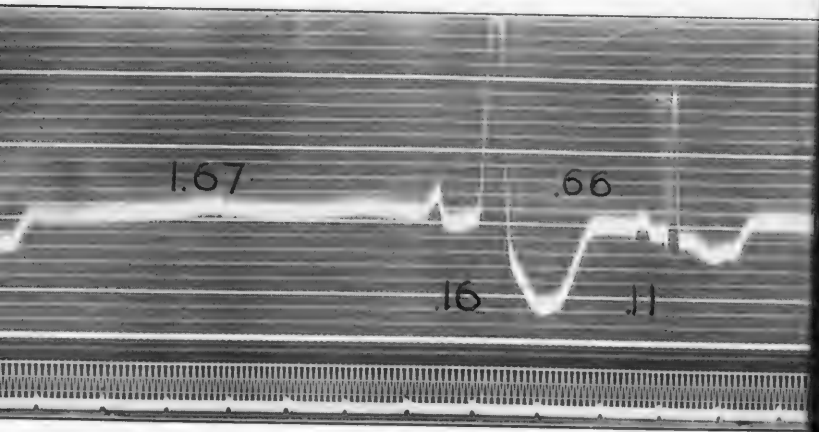


Fig. 14B.

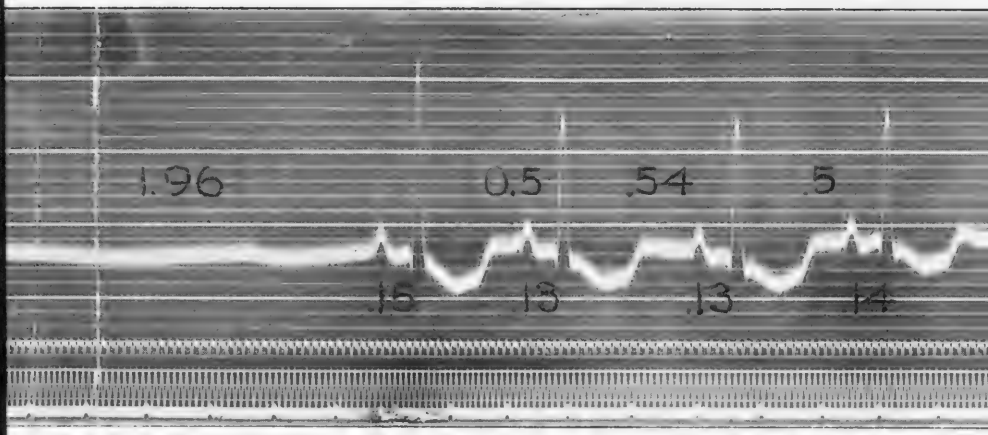


Fig. 14A.

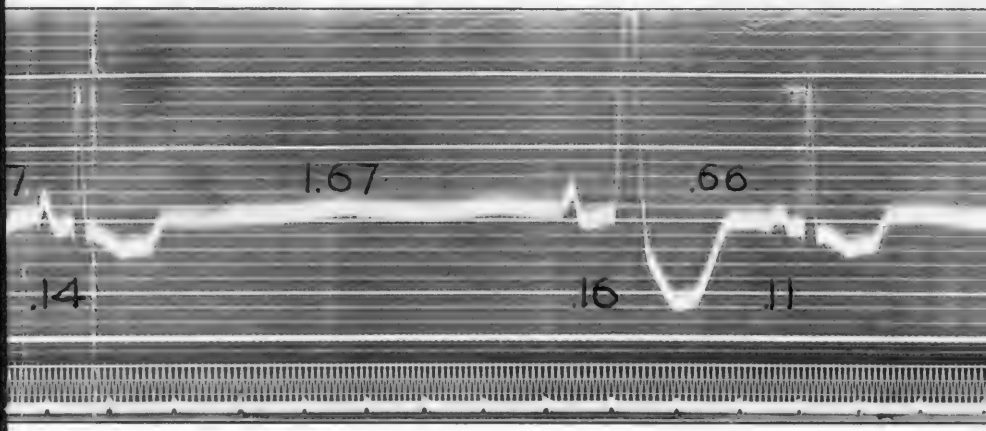
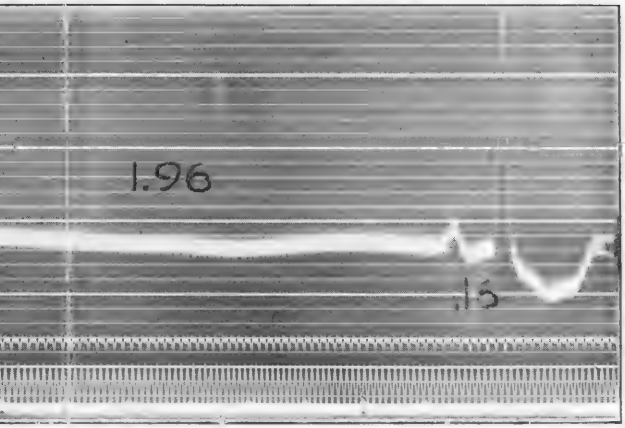
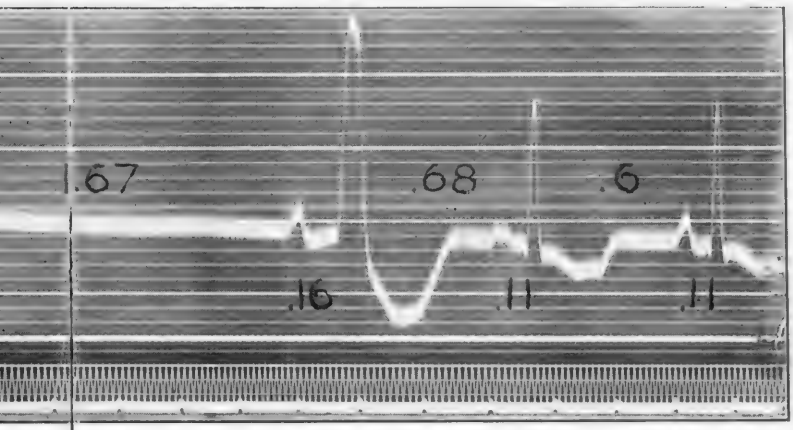


Fig. 14B.





COHN: Effects of Stimulation of Two Vagus Nerves.

43. Harrington, D. W., *Am. Jour. Physiol.*, 1898, i, 383.
44. Eppinger, H., and Rothberger, J., *Ztschr. f. klin. Med.*, 1910, lxx, 1.
45. Eppinger, H., and Stoerk, O., *Ztschr. f. klin. Med.*, 1910, lxxi, 157.
46. Engel, I., *Beitr. z. path. Anat. u. z. allg. Path.*, 1910, xlviii, 499.
47. Wilson, J. G., *Proc. Roy. Soc. London, Series B*, 1909, lxxxi, 151.
48. Kahn, R. H., *Arch. f. d. ges. Physiol.*, 1911, cxl, 627.
49. Kraus, F., and Nicolai, G., Das Elektrokardiogram, Leipzig, 1910.
50. von Tabora, D., *Ztschr. f. exper. Path. u. Therap.*, 1906, iii, 499.
51. Keng, Lim Boon, *Jour. Physiol.*, 1893, xiv, 467.
52. von Schumacher, S., *Sitzungsb. d. k. Akad. d. Wissensch., Math.-Naturwiss. Kl., Abt. III*, 1902, cxi, 133.

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice.*

By RICHARD M. PEARCE, M.D., J. H. AUSTIN, M.D., and JOHN H. MUSSER, Jr., M.D.
(*From the John Herr Musser Department of Research Medicine of the University of Pennsylvania, Philadelphia.*)

In the first paper[1] of this series certain phenomena have been described which follow splenectomy or are associated with the administration of a hemolytic serum to splenectomized animals. The most striking of these are (1) the frequent failure of jaundice to occur upon the administration of a hemolytic serum during the early period after splenectomy; (2) the increased resistance of the red blood corpuscles, which is evident about one month and more after splenectomy; and (3) the occasional occurrence of spontaneous jaundice in dogs splenectomized for several months. One possible explanation of the first of these phenomena—an influence of the spleen upon the alteration of hemoglobin or its transformation into bile pigment—has been shown, in the second paper[2] of the series, to be untenable. This investigation, moreover, brought out very definite and valuable information concerning the elimination of hemoglobin by the kidney, and the exact relation between hemoglobinemia and jaundice.

In the course of these studies, observations were made which

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, July 13, 1912.

[1] Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy, *Jour. Exper Med.*, 1912, xvi, 363.

[2] Pearce, R. M., Austin, J. H., and Eisenbrey, A. B., II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals, *Jour. Exper. Med.*, 1912, xvi, 375.

pointed strongly to changes in the blood itself as important factors in the explanation of the phenomena under investigation.

It was noticed, more or less accidentally, that an injection of hemolytic serum into anemic dogs, whether splenectomized or not, did not cause jaundice as readily as in dogs with normal red cell count and normal hemoglobin content.

This observation suggested that it might not be the mere absence of the spleen, but secondary changes in the blood consequent upon the absence of the spleen, that prevented the appearance of jaundice in splenectomized animals. We therefore turned our attention to the condition of the blood in all splenectomized animals. This phase of our problem has already been studied by one of us,[3] who found, in accord with other investigators, that in the dog splenectomy is followed by a gradual progressive decrease in red blood cells and hemoglobin reaching the lowest level about the twenty-sixth day, the hemoglobin varying from 50 to 65 per cent. and the red cells from 2,800,000 to 3,500,000. From this time to the eighty-second day a constant increase in hemoglobin and red cells occurs, the animal not reaching or approximating its normal level, however, until the end of about four and a half months.

A comparison of these phases of blood destruction and regeneration with the various phenomena we had observed was instructive. The period during which jaundice failed to follow hemolysis, or was slight and evanescent, corresponded roughly to the period, one month, of progressive blood degeneration; the period of increased resistance of the red cells corresponded to the period during which regeneration of blood was occurring, and spontaneous jaundice corresponded, apparently, to the period of more or less complete return of red cells and hemoglobin content to normal.

This correlation suggested the possibility that the failure of jaundice in the early period after splenectomy might be associated in some way with the low hemoglobin content of the red blood cells. Obviously it was possible to test this hypothesis by studying the effect of an hemolytic serum on dogs rendered anemic in some other way than by splenectomy. This was done by bleeding, as shown in

[3] Musser, J. H., Jr., An Experimental Study of the Changes in the Blood Following Splenectomy, *Arch. Int. Med.*, 1912, ix, 592.

the following experiment, in which a normal dog with high hemoglobin content is contrasted with a dog rendered moderately anemic by bleeding.

EXPERIMENT I.[4]

Effect of Hemolytic Serum on a Dog Rendered Anemic by Bleeding. Normal Control.

Date.	Anemic dog.	Date.	Control dog.
April 18, 1912.	Weight, 10,740 gm.	April 16, 1912.	Weight, 5,350 gm.
	Urine: free of albumin.		Urine: no albumin, no bile.
	179 c.c. of blood taken from jugular vein.		Blood: red cells, 5,390,000; hemoglobin 107 per cent.
April 20, 1912.	150 c.c. of blood taken from jugular vein.		
April 21, 1912,			Fragility: 0.4+; 0.5−[5].
10 A. M.	Urine: no albumin, no bile pigment.	11.45 A. M.	Received in vein 0.25 c.c. per kilo of same serum as anemic dog.
	Blood: red cells, 4,450,000; hemoglobin 87 per cent.	2.45 P. M.	Much bile in urine.
	Fragility: 0.3+; 0.4−[5].	3.15 P. M.	Blood: red cells, 5,330,000; hemoglobin 98 per cent.
April 21, 1912, 11.10 A. M.	Received 0.25 c.c. hemolytic serum per kilo, in vein.	6.30 P. M.	Urine: contains hemoglobin and much bile.
5 P. M.	Urine: no albumin, no bile.		Blood: red cells, 4,660,000; hemoglobin 85 per cent.
	Blood: red cells, 3,400,000; hemoglobin 54 per cent.		
April 22, 1912.	Urine: no hemoglobin, no bile.	April 17, 1912.	Urine contains hemoglobin and bile pigment.
	Blood: red cells, 3,250,000; hemoglobin 49 per cent.		Blood: red cells, 4,470,000; hemoglobin 73 per cent.
April 23, 24, 25, 1912.	Urine: no bile pigment.		
April 24, 1912.	Blood: red cells, 3,040,000; hemoglobin 42 per cent.	April 18, 1912.	Urine: no hemoglobin, but trace of bile.
April 25, 1912.	Blood: red cells, 2,910,000; hemoglobin 42 per cent.	April 19, 1912.	Urine: no bile, no albumin.
			Blood: red cells, 4,930,000.

In this experiment the anemic dog although it received the same amount of serum proportionately, and on account of its greater weight, twice as much serum actually as the control, failed to develop hemoglobinuria or jaundice. It is true that in as far as the salt solution test can be taken as an indicator, the corpuscles of this

[4] In the experiments described in this paper, all operative procedures were carried out under complete ether anesthesia.

[5] These figures refer to hemolysis in different strengths of salt solution; thus 0.3+ indicates complete hemolysis in 0.3 per cent. salt solution; 0.4— indicates no hemolysis in 0.4 per cent. solution, and that partial hemolysis occurred in 0.35 per cent. solution.

dog were apparently more resistant than those of the control dog. Nevertheless, the lower hemoglobin content of the blood would appear to be an important factor in determining the occurrence of jaundice. It was at once evident that if it is the condition of the blood and not the absence of the spleen that is important, a recently splenectomized dog with the normal number of red cells and normal hemoglobin content should develop jaundice after the administration of an hemolytic serum. Such a dog was at hand at the time the above experiments were made and was injected with the same serum used in experiment I. As a control, a dog was used that had been splenectomized sixty-six days previously and had recovered from the anemia following splenectomy.

EXPERIMENT II.

Effect of Hemolytic Serum on Splenectomized Dogs with Normal Red Cell Count.

Date.	Four day splenectomy.	Date.	Sixty-six day splenectomy.
April 17, 1912.	Weight, 7,850 gm. Splenectomy.	Feb. 10, 1912.	Splenectomy.
		April 16, 1912.	Weight, 12,680 gm.
April 21, 1912.	Urine: no albumin, no bile. Blood: red cells, 5,880,000; hemoglobin 102 per cent. Fragility: 0.3+; 0.45−.		Urine: faint trace of albumin, no bile. Blood: red cells, 5,230,000; hemoglobin 83 per cent. Fragility: 0.25+; 0.35−.
11.25 A. M.	Received in vein 0.25 c.c. serum per kilo (same serum as in experiment I).	12 M.	Received in vein 0.25 c.c. serum per kilo (same serum as in experiment I).
5.20 P. M.	Urine: (by catheter) contains large amount of bile pigment and faint trace of albumin. Blood: red cells, 5,330,000; hemoglobin 93 per cent.	2.45 P. M.	Urine: trace of bile pigment.
		3.50 P. M.	Blood: red cells, 5,310,000; hemoglobin 84 per cent.
April 22, 1912.	Urine: trace of bile. Blood: red cells, 5,260,000; hemoglobin 81 per cent.	11.00 P. M.	Urine: moderate amount of bile pigment.
April 23, 1912.	Urine: faint trace of bile.		
April 24, 1912.	Urine: no albumin, no bile. Blood: red cells, 4,800,000; hemoglobin 70 per cent.	April 17, 1912.	Urine: moderate amount of bile pigment. Blood: red cells, 4,830,000; hemoglobin 79 per cent.
April 25, 1912.	Blood: red cells, 4,510,000; hemoglobin 80 per cent.	April 18, 1912.	Urine: no bile pigment. Blood: red cells, 4,500,000.
		April 19, 1912.	Urine: no bile.

If the results in these four animals (experiments I and II) are compared, it is seen that jaundice failed to occur only in the animal

EXPERIMENT III.

Anemic Splenectomized Dog Controlled by Splenectomized Dog with Normal Blood.

Date.		Date.	
June 2, 1912.	Splenectomy.	May 7, 1912.	Splenectomy.
June 5, 1912.	Blood: red cells, 4,750,000; hemoglobin 82 per cent.	June 5, 1912.	Blood: red cells, 5,230,000; hemoglobin 90 per cent.
	Urine: normal, no bile pigment.		Urine: normal, no bile pigment.
	1.75 c.c. hemolytic serum injected into vein.		1.75 c.c. of same serum injected into vein.
June 6, 1912.	Urine: hemoglobin present, no bile pigment.	June 6, 1912.	Urine: hemoglobin present, bile pigment abundant.
June 7, 1912.	Urine: hemoglobin present, no bile pigment.		
	Died.	June 7, 1912.	Urine: hemoglobin present, bile pigment abundant.
			Experiment discontinued.

rendered anemic by bleeding; both splenectomized animals with normal red cell count had a transient but definite elimination of bile pigment in the urine, while the normal control exhibited both hemoglobinuria and jaundice. These observations are of interest in connection with our studies in the second paper of this series. The normal dog suffered a destruction of red cells and reduction of hemoglobin equal to 22 per cent. of the original hemoglobin content. This destruction, as shown by the appearance of hemoglobin in the blood serum two hours and forty minutes after injection, was severe and rapid. The threshold value of the kidney was soon reached and hemoglobin appeared in the urine, but at the same time the amount going to the liver and transformed into bile pigments exceeded the capacity of that organ and resorption caused the pigment to appear in the urine. In the two splenectomized animals, the destruction of hemoglobin and red cells was less, 6 and 16 per cent. respectively, and apparently too slow to cause a concentration of hemoglobin in the blood sufficient to reach the threshold value of the kidney; hemoglobinuria therefore did not occur, but the amount going to the liver was sufficient to lead to jaundice. The slight destruction in the dog that had been splenectomized sixty-six days before is easily explained by increased resistance of the corpuscles; the loss being only 6 per cent. as compared with the anemic dog in

which the loss was 28 per cent., though both had, at the time of injection, practically the same percentage of hemoglobin. The one thing that stands out prominently in these four experiments is the fact that jaundice failed in the dog rendered anemic by bleeding, but occurred in each of the other three dogs with high red cell count.

That this holds true also for the splenectomized anemic dog is shown by experiment III, in which a dog, moderately anemic, three days after splenectomy is controlled by a non-anemic dog which had been splenectomized twenty-six days before.

Here a relatively slight difference in the red cell count and hemoglobin content appears to have been the chief factor in the non-appearance of jaundice.

Another observation, an account of which follows, adds further evidence to support the theory that an hemolytic serum causes jaundice only when the splenectomy is not followed by anemia.

EXPERIMENT IV.

A dog weighing 7,730 gm. was splenectomized on March 11. Three days later the urine was normal, the red cells numbered 6,210,000, the hemoglobin content was 92 per cent., and the fragility test gave complete hemolysis in 0.4 per cent. solution. At this time the animal received intravenously 0.5 c.c. per kilo of a very powerful serum, which caused death in less than forty-eight hours. Hemoglobinemia was evident one hour and twenty minutes after injection and hemoglobinuria after four hours; at this time the hemoglobin had dropped to 80 per cent. and at the end of twenty-four hours to 57 per cent. The severe hemoglobinuria rendered tests for bile pigment difficult of interpretation but at autopsy intense bile staining of the skin, sclera, and the mucous membranes and other lighter tissues of the body was found. The abdominal cavity contained a small amount of blood, and petechial hemorrhages studded the peritoneum. The liver showed diffuse necroses and the gall bladder was distended by a thick, black, mush-like bile which would not flow through an incision made in the gall bladder. The lymph nodes of all parts of the body were hemorrhagic. The blood serum contained free hemoglobin.

Another similar experiment follows.

EXPERIMENT V.

Effect of Hemolytic Serum Nine Days after Splenectomy, in the Presence of Normal Red Cell Count.

Date.	Dog 28, weight 10,015 gm.	Date.	Dog 37, weight 8,255 gm.
March 5, 1912.	Urine normal.		
March 6, 1912.	Splenectomy.		Control.
March 8, 1912.	Blood: red cells, 7,620,000; hemoglobin 102 per cent.		
March 13, 1912.	Blood: red cells, 6,540,000; hemoglobin 105 per cent. Fragility: 0.25+; 0.35−.		
March 14, 1912.	Urine: faint trace of albumin, no bile pigment.	March 15, 1912.	Urine: normal; no bile pigment.
March 15, 1912.	Urine: faint trace of albumin, no bile pigment.	3.15 P. M.	Blood: red cells, 5,440,000; hemoglobin 90 per cent.
1.30 P. M.	Blood: red cells, 6,260,000; hemoglobin 98 per cent.		Fragility: 0.35+; 0.45−.
1.55 P. M.	0.3 c.c. serum per kilo in vein.	3.30 P. M.	0.3 c.c. of same serum per kilo in vein.
3.35 P. M.	Blood serum clear.		
5.15 P. M.	Urine: by catheter, deeply bile stained, considerable coagulable protein; no hemoglobin.	4.05 P. M.	Blood serum free from hemoglobin.
		5.45 P. M.	Urine by catheter deeply bile stained, and contains much coagulable protein.
	Red cells: 5,490,000; hemoglobin 100 per cent.		
	Blood serum: clear.		Blood: red cells, 5,720,000; hemoglobin 85 per cent.; serum shows doubtful trace of hemoglobin.
March 16, 1912, 9 A. M.	Urine: flocculent precipitate of albumin, large amount of bile pigment; no hemoglobin.		
2 P. M.	Urine: no hemoglobin, much bile pigment, albumin present.		Urine: highly colored by bile pigment, trace of albumin; no hemoglobin.
3.30 P. M.	Red cells, 3,600,000; hemoglobin 72 per cent.	March 16, 1912, 5.45 P. M.	Red cells, 4,480,000; hemoglobin 67 per cent.
March 17, 1912.	Urine: no hemoglobin, bile present, albumin present.		Urine: bile pigments lessening.
3 P. M.	Blood: hemoglobin 50 per cent.		Blood: hemoglobin 52 per cent.
March 18, 1912.	Urine: trace of albumin, bile pigments diminishing in amount.		Urine: faint trace of bile pigment.
	Blood: hemoglobin 37 per cent.		Blood: red cells, 2,320,000; hemoglobin 45 per cent.
March 19, 1912.	Blood: hemoglobin 40 per cent.; red cells, 3,170,000.		Urine: faint trace of bile.
	Serum shows no free hemoglobin.		Blood: red cells, 3,020,000; hemoglobin 40 per cent.
	Urine: high colored, no albumin, good reaction for bile pigments.		Blood: serum contains no free hemoglobin.
March 20–27, 1912.	Urine: contains bile pigment in small amounts.	March 20 and 21, 1912.	Urine: contains trace of bile pigments and albumin.
March 21, 1912.	Blood: red cells, 2,350,000; hemoglobin 31 per cent.		

EXPERIMENT V (*Continued*).

Date.	Dog 28, weight 10,015 gm.	Date.	Dog 37, weight 8,255 gm.
March 23, 1912.	Blood: red cells, 2,500,000; hemoglobin 35 per cent. Fragility: 0.25+; 0.35−.	March 21, 1912.	Blood: red cells, 3,040,000; hemoglobin 33 per cent.
March 26, 1912.	Blood: r d cells, 3,260,000; hemoglobin 51 per cent. Experiment discontinued.	March 23, 1912.	Urine no albumin, no bile pigment. Died.

SPLENECTOMIZED ANIMALS WITH INCREASED RESISTANCE OF CORPUSCLES.

That the red cells of splenectomized animals after one month and more have a true resistance to hemolytic serum and that this is not merely a matter of test-tube experiment is shown in experiment VI.

EXPERIMENT VI.

Increased Resistance Forty Days after Splenectomy.

Date.		Date.	
Feb. 8, 1912.	Splenectomy.		Control.
March 11, 1912.	Weight, 6,780 gm. Urine: normal. Blood: red cells, 5,560,000; hemoglobin 65 per cent. Fragility: 0.3+; 0.4−.	March 11, 1912.	Urine: normal.
March 19, 1912.	Urine: normal. Blood: red cells, 5,710,000; hemoglobin 82 per cent.		Blood: red cells, 6,220,000; hemoglobin 102 per cent.
4.30 P. M.	0.5 c.c. serum per kilo in vein.		Fragility: 0.4+; 0.5−.
6 P. M.	Serum faintly tinged with hemoglobin.	2.35 P. M.	0.5 c.c. per kilo of same serum in vein.
9.30 P. M.	No urine in bladder. Blood: red cells, 5,550,000; hemoglobin 65 per cent.	4.02 P. M.	Serum tinged with hemoglobin.
		6.30 P. M.	No urine in bladder. Serum deeply stained with hemoglobin.
March 20, 1912.	Died. Urine from bladder shows much albumin, trace of bile; no hemoglobin.	12 P. M.	Red cells, 4,330,000; hemoglobin 94 per cent. Died during night; no urine.

These animals died as the result of a toxic property distinct from the hemolytic activity of the serum, but the difference in the resistance of the red cells to the hemolytic agent is clearly shown.

Still more striking is the very slight destruction of red cells in an animal that developed spontaneous jaundice seven and a half months after splenectomy, as shown in experiment VII.

EXPERIMENT VII.

Increased Resistance Seven and a Half Months after Splenectomy.

Date.	Dog 32,[6] weight 9,170 gm.	Date.	Dog 33, weight 7,050 gm
July 19, 1911.	Splenectomy.		Control.
March 7, 1912.	Much bile pigment in urine.		Urine: normal.
March 8, 1912,	Much bile pigment in urine.		Urine: normal.
11 A. M.	Blood: red cell count 6,120,000; hemoglobin 97 per cent.	10.45 A. M.	Blood: red cell count, 6,610,000; hemoglobin 108 per cent.
2.45 P. M.	Red cells hemolyzed completely by 0.3 per cent. salt solution.		
2.47 P. M.	0.5 c.c. serum per kilo in vein.	2.16 P. M.	Red cells hemolyzed completely by 0.4 per cent. salt solution.
3.02 P. M.	No free hemoglobin in serum.		
3.18 P. M.	No free hemoglobin in serum.		
3.38 P. M.	No free hemoglobin in serum.		
5.15 P. M.	Urine free of hemoglobin.	2.18 P. M.	0.5 c.c. of same serum per kilo in vein.
5.30 P. M.	Blood count: red cells, 6,480,000; hemoglobin 101 per cent.		
March 9, 1912.	Urine: no hemoglobin, but much bile pigment.	2.32 P. M.	No free hemoglobin in serum.
2.15 P. M.	Blood count 6,800,000; hemoglobin 102 per cent.	2.53 P. M.	Trace of hemoglobin in serum.
2.30 P. M.	Received in vein 8.5 c.c. of same serum (nearly 1 c.c. per kilo).	3.10 P. M.	Trace of hemoglobin in serum.
2.32 P. M.	No hemoglobin in serum.	5.10 P. M.	Urine contains hemoglobin.
2.55 P. M.	No hemoglobin in serum.		
5.00 P. M.	Trace of hemoglobin in serum.	5.10 P. M.	Blood count, 3,580,000; hemoglobin 103 per cent.
5.00 P. M.	Hemoglobin in urine in very small amount but positively determined by spectroscope.	March 9, 1912.	Urine: very marked hemoglobinuria: bile test positive.
5.00 P. M.	Red cells, 5,200,000; hemoglobin 95 per cent.		No second injection.
March 10, 1912.	Marked hemoglobinuria.		
March 11, 1912.	Died.	March 11, 1912.	No hemoglobin in urine, but trace of bile.

Here again the increased resistance of the red cells of the splenectomized animal is very marked. Equal doses of serum failed to cause marked hemolysis in the splenectomized animal, as determined by blood count and absence of hemoglobin in serum and urine. An extraordinarily large dose, however, caused severe blood destruction and death after forty-eight hours. On the other hand, the first dose caused in the control animal marked hemoglobinuria and great destruction of red cells.

[6] One of the dogs (No. 2) used by J. H. Musser, Jr., in his "Experimental Study of the Changes in the Blood Following Splenectomy," *Arch. Int. Med., loc. cit.*

THE CONDITION OF THE BLOOD IN ANIMALS WITH SPONTANEOUS JAUNDICE.

The observation that animals with low red cell count and low hemoglobin, irrespective of the presence or absence of the spleen, failed to develop jaundice after the administration of a hemolytic serum, and the obverse, that this serum in splenectomized animals with normal blood count and hemoglobin content always caused jaundice, suggested the possibility that the spontaneous jaundice, occasionally occurring after splenectomy, might be associated in some way with an over-regeneration of blood leading to an abnormally high red cell count and hemoglobin content. With this possibility in view blood examinations have been made in all long period experiments. The data at hand, though meager, are suggestive of a possible relation between the jaundice and the condition of the blood.

Well marked choluria following splenectomy, and occurring without administration of a hemolytic poison, has been seen in two animals, and slight choluria likewise in two. In one of the former the blood was not examined; in the second (experiment VII), in which a very marked choluria was found seven and a half months after operation, the red cell count was 6,120,000, and the hemoglobin 97 per cent. So also in one of the animals with slight choluria, the blood count was 6,220,000, and hemoglobin 103 per cent.; in a second the red count was 5,740,000.

These were animals which had recovered from the temporary anemia due to splenectomy and possessed the normal number, or perhaps a slight excess of red blood cells. On the other hand, in the only animal which was carried for a long period (ten months) with careful observation of the blood and which did not develop choluria, the blood counts were low, varying during the last two months from 4,460,000 to 4,970,000, and with hemoglobin ranging from 68 to 81 per cent.

These observations are too few in number and not sufficiently controlled to allow definite conclusions but they suggest the possibility that spontaneous jaundice after splenectomy may be associated in some way with the period of complete regeneration of the blood.[7]

[7] This suggestion we offer with some hesitancy, for recently we have observed in five apparently normal dogs an unexplainable spontaneous jaundice.

SUMMARY.

The results of this study of the changes in the blood after splenectomy and of the effect of the administration of hemolytic serum allow the following tentative conclusions:

1. The failure of a hemolytic serum to cause jaundice is due in some way to the anemia which frequently follows splenectomy. Animals, whether splenectomized or not, which have a low blood count and low hemoglobin content do not readily develop jaundice after administration of a hemolytic serum. On the other hand, in animals with a normal blood picture such a serum readily causes, during early periods after splenectomy, a well marked jaundice.

2. The difficulty of producing hemoglobinemia and jaundice in animals splenectomized one month or more, is due, as shown by blood count, the use of hypotonic salt solution test, and by the search for the presence of hemoglobin in the serum and urine, to an increase in the resistance of the red cells. The results of detailed serologic tests upon this point will be presented later.[8]

3. It is possible that spontaneous jaundice occurring at long periods after splenectomy is an accompaniment of the complete regeneration of the blood. The study of the blood in animals splenectomized for long periods, seven to ten months, indicates that spontaneous jaundice occurs only when the animal has recovered from the initial period of anemia and has a high red cell count and high hemoglobin content.

4. As to the mechanism responsible for the anemia following splenectomy and for the increased resistance of the red cells, we can at present offer no explanation.

[8] Karsner, H. T., and Pearce, R. M., IV. A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood Corpuscles after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 769.

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

IV. A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood Corpuscles after Splenectomy.*

By HOWARD T. KARSNER, M.D., and RICHARD M. PEARCE, M.D.

(From the John Herr Musser Department of Research Medicine of the University of Pennsylvania, Philadelphia.)

In the first and third communications of this series[1] it was shown that a dog that has been splenectomized for some time is more resistant to a specific hemolytic immune serum than is the non-splenectomized dog, and that to produce hemoglobinemia and hemoglobinuria, or jaundice, in such an animal it is necessary to administer larger amounts of serum than in the normal animal. Tests *in vitro* with hypotonic salt solution and with hemolytic serum showed that this diminished susceptibility of the animals to the action of a hemolytic serum was in all probability due to an increased resistance of the red cells. These tests, however, were made irregularly and were but incidental to other phases of the general investigation. In the present communication the results of a special study of this problem by the accurate and detailed methods of immunology are presented in detail.

Increase in resistance of the red cells to hemolytic agents after splenectomy is not a new observation. It has been noted in the dog by Bottazzi,[2] Banti,[3]

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, July 13, 1912.

[1] Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 363; Pearce, R. M., Austin, J. H., and Eisenbrey, A. B., II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals, *ibid.*, 375; Pearce, R. M., Austin, J. H., and Musser, J. H., Jr., III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice, *ibid.*, 758.

[2] Bottazzi, F., La milza come organo emocatatonistico, *Sperimentale, Sez. biol.*, 1894, xlviii, 433.

[3] Banti, G., La milza nelle itterizie pleiochromiche, *Gaz. d. osp.*, 1895, xvi, 489.

Vast,[4] Pugliese and Luzzatti,[5] and Joannovics;[6] in the guinea pig by Gabbi,[7] and in the rabbit by Domenicci.[8] The hemolytic agent used by these investigators was, as a rule, toluylendiamine, though occasionally pyrodin (Pugliese and Luzzatti), acetylphenylhydrazine (Banti), and specific hemolytic immune serum (Joannovics) have been employed. In general it has been assumed that the increased resistance to the toxic influence of the drug, or the failure to produce hemoglobinemia or jaundice, is due to increased resistance of the red cells. Banti invokes also the absence of the alleged hemolytic function of the spleen, and Vast also believes that for the destruction of red cells the intervention of the spleen is necessary. This point of view is to some extent supported by Joannovics. Pugliese and Luzzatti, on the other hand, deny the influence of the absence of the spleen and point to the presence, after repeated doses of pyrodin, of newly formed immature nucleated corpuscles, poor in hemoglobin, which they think may possibly be more resistant to pyrodin than are the normal cells. Few exact studies of the condition of the corpuscles have been made, and these are limited, as in the investigations of Brissaud and Bauer,[9] Chalier and Charlet,[10] and Pel,[11] to the use of hypotonic salt solutions. No careful study of the resistance of these cells to hemolytic serum *in vitro* has been made, and the allied problems of possible antihemolytic action of the serum and of possible changes in complement content have not been investigated.

Brissaud and Bauer, working with two rabbits and using hypotonic salt solution of varying strengths, found a decreased resistance of the red cells during eight to ten days after splenectomy with, "after the lapse of time," a return to normal, but no increase in resistance. They do not, however, state how long the experiment was continued. Chalier and Charlet, using both the rabbit and the dog, and the salt solution test, conclude that splenectomy is followed apparently by a slight increase in the resistance of the red cells. Pel, in a very extensive study, found that as an average of fifty-eight determinations on normal dogs the first trace of hemolysis occurred in 0.42 per cent. salt solution as compared with 0.35 as the average for thirty observations on splenectomized dogs; the average concentration at which hemolysis was complete was, for normal dogs, 0.30 per cent. salt solution, and for splenectomized dogs 0.23 per

[4] Vast, cited by Joannovics, *loc. cit.;* original not available.

[5] Pugliese, A., and Luzzatti, T., Contribution à la physiologie de la rate, *Arch. ital. de biol.*, 1900, xxxiii, 349.

[6] Joannovics, G., Experimentelle Untersuchungen über Ikterus, *Ztschr. f. Heilk., Abt. f. path. Anat.*, 1904, xxv, 25.

[7] Gabbi, U., Ueber die normale Hämatolyse mit besonderer Berücksichtigung der Hämatolyse in der Milz, *Beitr. z. path. Anat. u. z. allg. Path.*, 1893, xiv, 351.

[8] Domenicci, cited by Pel, *loc. cit.;* original not available.

[9] Brissaud and Bauer, Recherches sur la résistance des globules rouges chez le lapin, *Compt. rend. Soc. d. biol.*, 1907, lxii, 1068.

[10] Chalier, J., and Charlet, L., État de la résistance globulaire chez l'animal normal et splenéctomisé, *Jour. de physiol. et de path. gén.*, 1911, xiii, 728.

[11] Pel, L., Über die Resistenz der roten Blutkörperchen gegenüber hypotonischen Kochsalzlösungen bei entmilzten Hunden, *Deutsch. Arch. f. klin. Med.*, 1912, cvi, 592.

cent. Thus in both series of observations the increased resistance of the splenectomized animals was the equivalent of 0.07 per cent. salt solution. The difference may be expressed in another way; in the fifty-eight observations on normal animals hemolysis began in all but one test in solutions of 0.48 to 0.40 per cent., while of thirty observations on splenectomized animals, in all but two it began in solutions of 0.38 to 0.30 per cent.; likewise complete hemolysis occurred in the normal group only seven times below 0.30, while in the splenectomized group it occurred always below 0.30 per cent. solution. The resistance of the red cells was found to increase gradually and reach its maximum after about two months; further lapse of time showed no tendency to return to normal. The longest period after splenectomy was two years and four months.

Pel makes a general statement concerning the influence of the serum to the effect that the serum of a splenectomized dog added to the red cells of a normal dog does not increase the resistance of the latter to hypotonic salt solution, and *vice versa*, that the addition of normal serum to the red cells of a splenectomized dog does not decrease their resistance.

Blood counts showed a slight decrease in the number of red cells after splenectomy but not enough in the opinion of Pel, in view also of only slight changes in the percentage of hemoglobin, to have any relation to the increased resistance of the red cells. As to the factors responsible for the increased resistance, Pel offers no explanation.

EXPERIMENTAL PART.

In the present investigation we have used six dogs. One had been splenectomized ten days, a second, thirty days, and a third, four months before. The last animal had been given hemolytic immune serum two months before the beginning of the immunological tests, and therefore as a control for this animal, a dog which had received hemolytic immune serum five weeks previously, but was otherwise normal, was included in the series, and as controls for the other two animals two normal dogs were used.

For the determination of the resistance of the corpuscles to hypotonic salt solution we employed, with slight modifications, the method of Theobald Smith[12] as used by Gay.[13] Chemically pure sodium chloride was dried for two hours at 170° C. and immediately weighed in amounts necessary to make 500 cubic centimeter volumes of salt solution ranging from 0.1 to 0.5 per cent. in steps of 0.025 per cent. In order to be sure of approximately the same

[12] Smith, T., and Brown, H. R., The Resistance of the Red Corpuscles of the Horse to Salt Solutions of Different Tonicities, *Jour. Med. Research*, 1906, xv, 425.

[13] Gay, F. P., The Function of Tonicity in Human Isohemagglutination, *Jour. Med. Research*, 1907–08, xvii, 321.

TABLE I.

	Percentage strength of salt solutions. (June 10, 1912)	0.275	0.300	0.325	0.350	0.375	0 400	0.425	0.450	0.475	0.500	0.525
Corpuscles of	Dog 30. Normal.....	100	100	100	100	100	100	100	60	40	Trace	0
	Dog 53. Normal.....	100	100	100	100	100 —	80	40	20	Trace	0	0
	Dog 51. Splenectomy, May 31.....	100	100 —	100 —	80	60	20	Trace	Trace	0	0	0
	Dog 46. Splenectomy, May 10.....	100	100	100 —	100 —	80	60	40	20	Trace	0	0
	Dog 24. Splenectomy, Feb. 10. Immune serum, April 16.....	100	100 —	80	40	20	Trace	Trace	0	0	0	0
	Dog. 43 Immune serum, April 21.....	100	100	100	100	80	60	20	Trace	0	0	0

volume of corpuscles in the anemic as in the normal bloods, the gently defibrinated blood was centrifuged and the serum drawn off. 0.1 of a cubic centimeter of the corpuscular mass was measured accurately in a graduated pipette and placed in three cubic centimeters of each of the various salt solutions. Standard colorimetric scales for comparison were made by laking red cells with distilled water; thus the laking of 0.4 of a cubic centimeter of the corpuscular mass in twelve cubic centimeters of distilled water represented a standard of 100 per cent. hemolysis. Dilutions of this solution were made so as to have tubes showing the color values of 80, 60, 40, and 20 per cent. hemolysis. Less than 20 per cent. hemolysis is indicated in table I as a trace of hemolysis. In most instances this scale was entirely satisfactory, but occasionally, although a tube showed 100 per cent. hemolysis colorimetrically, there was, on shaking, a slight macroscopic sediment of incompletely hemolyzed corpuscles; this result is indicated by a minus sign after the approximate percentage of hemolysis. Upon adding the corpuscles to the salt solution, a preliminary reading was made and the mixtures were placed in the refrigerator. The final reading at the end of eighteen hours was made the basis for table I.

It will be seen that the blood of the normal dogs (30 and 53) shows hemolysis in fairly high percentages of salt solution, but that the resistance is increased in all the abnormal animals. It is true that dog 46, a splenectomized animal, shows initial hemolysis in the same percentage of salt solution as normal dog 53; but inspection will show that whereas in the normal dog hemolysis is complete at 0.350 per cent., it is not complete in dog 46 until 0.300 per cent. is reached. There can be no doubt that the cells of dog 24, the animal which had been longest splenectomized, show the greatest degree of resistance. That this resistance is due for the most part, if not entirely, to splenectomy, is, in view of results with bloods 30 and 53, most probable. On the other hand, it is evident, as shown by the experience with blood 43, that the administration of a hemolytic immune serum is followed by an increased resistance of the red cells in the absence of splenectomy.

In order to determine the resistance of the corpuscles to a specific

hemolytic immune serum[14] the following technique was employed. The corpuscles were washed three times in 0.85 per cent. salt solution, and blood suspensions were made of 5 per cent. of red cells as contained in the centrifuged corpuscular mass. The latter rather than whole blood was used because the use of whole blood would be fallacious in the case of anemic animals. The immune serum was titrated against normal corpuscles, guinea pig complement was used in doses of 0.1 of a cubic centimeter, and the experiment arranged as indicated in table II.

TABLE II.

Washed corpuscle suspensions of	Dilutions of immune serum. (June 14, 1912)	1/20	1/50	1/100	1/150	1/200	1/250	1/300	Complement control.	Amboceptor control.	Blood control.
Dog 30.	Normal.....	C.H.[15]	C.H.	H.	H.	P.H.	P.H.	P.H.	0	0	0
Dog 53.	Normal.....	C.H.	C.H.	H.	H.	P.H.	P.H.	P.H.	0	0	0
Dog 51.	Splenectomy, May 31 ..	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0
Dog 46.	Splenectomy, May 10 ..	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0
Dog 24.	Splenectomy, Feb. 10. Immune serum, April 16 ..	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0	0	0
Dog 43.	Immune serum, April 21 ..	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0

Technical limitations prevent, in this experiment, as close an estimation of resistance as is possible with hypotonic salt solution, but it can be seen readily that whereas dilutions of 1/20 and 1/50 produced complete hemolysis of normal corpuscles, the corpuscles of the abnormal animals were resistant to these dilutions. The fact that the corpuscles of dog 24, which had been splenectomized four months previously, were most resistant is shown by the fact that whereas partial hemolysis appeared in all other corpuscles in dilutions of 1/250 and 1/300, the corpuscles of this dog resisted completely hemolysis at such dilutions. Here again the results with the blood of dog 43 demonstrate that the administration of an hemolytic

[14] Prepared by injecting the rabbit five times with 5 c.c. of dog blood, at intervals of five to seven days.

[15] In the tables, C.H. = complete hemolysis; H. = hemolysis; P.H. = partial hemolysis.

serum increases the resistance of the red cells, irrespective of splenectomy.

Since it has been shown by Karsner and Pearce[16] that agglutination limits hemolysis, it was considered wise to avoid possible error due to such action. The agglutinative effect of the immune serum was therefore examined in the same dilutions as were used for hemolysis. It was found that the agglutinability of the corpuscles was the same for all bloods except that of dog 43, the corpuscles of which formed somewhat smaller and less firm clumps than the others, but agglutinated at the same time and in the same dilutions as the rest. It was thus conclusively shown that the variations in resistance to hemolysis do not depend on variability of agglutination.

The fact that washed corpuscles were used in the experiments with immune serum would indicate that the increase in resistance is indeed a property of the corpuscles themselves, but in order to prove this point absolutely, the sera of all six dogs were examined for antihemolytic properties. It has been shown by Karsner and Pearce[17] that fresh dog serum has a definite antihemolytic property in an homologous hemolytic system. In the experiments designed to determine whether this property is augmented after splenectomy, an immune serum was carefully titrated with a constant dose of guinea pig complement. Two rows of tubes were used; in the tubes of one row were placed 0.5 of a cubic centimeter of a dilution of dog serum, as indicated in table III, and one dose (0.5 of a cubic centimeter) of amboceptor. In the second row the same amount of dog serum and two doses of amboceptor were placed. The tubes were then incubated for one half hour, and to the first row one dose of complement was added, to the second row two doses of complement, and to all the tubes the proper amount of 5 per cent. blood suspension. The second incubation lasted one hour and the readings were found to be uniform throughout. The results are shown in table III, in which, however, in order to avoid unnecessary duplication, the results with two sera only are given.

[16] Karsner, H. T., and Pearce, R. M., The Antibodies Produced by Various Constituents of Dog's Bile, *Jour. Med. Research*, 1912, xxvi, 357.

[17] Karsner, H. T., and Pearce, R. M., *loc. cit.*

TABLE III.

Serum dilutions. (June 15, 1912)		1/1	1/2	1/4	1/8	1/16	1/32	1/64	0
Serum of dog 30 (normal) in dilutions indicated plus	1 dose complement 1 dose amboceptor	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.
	2 doses complement 2 doses amboceptor	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
Serum of dog 24 (splenectomy Feb. 10, 1912. Immune serum April 16) in dilutions indicated plus	1 dose complement 1 dose amboceptor	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	C.H.
	2 doses complement 2 doses amboceptor	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.

It is seen that in all the dilutions of dog serum used, the action of one dose of amboceptor and of complement was hindered, but in none of the dilutions was the antihemolytic property sufficient to hinder the action of two doses of complement and of amboceptor. Weaker dilutions were not considered necessary because it seems certain that between the action of a dilution of 1/64 on one dose of hemolysin and of whole serum on two doses, no fine gradation could exist.

In determining the complement value of dog serum, it was thought best to use a sheep hemolytic system. The anti-sheep amboceptor was titrated with normal dog complement and the approximate complement value determined. Then two series of tubes were arranged with one dose of amboceptor, the proper amount of 5 per cent. sheep blood suspension, and in the first row increasing doses of fresh serum from the six dogs. The second row was placed so that each tube contained corresponding ingredients to those in the first row except that no anti-sheep amboceptor was present. The latter series showed the natural amboceptor in the dog serum. It was found that the natural sheep hemolysin was the same in all except one. In spite of this the complement value was precisely the same in all six animals. The results with one animal are charted (table IV) to show the technique.

TABLE IV.

Dog complement in c.c. (June 15, 1912)		0.01	0.02	0.04	0.07	0.1	1.5
Dog (normal) ..	with amboceptor.....	0	P.H.	H.	C.H.	C.H.	C.H.
	without amboceptor.....	0	0	Trace	H.	C.H.	C.H.

DISCUSSION.

These studies demonstrate conclusively that the increased resistance of the erythrocytes of the splenectomized dog depends upon the corpuscles rather than the serum. Pel's experiments demonstrated this fact in so far as the resistance to hypotonic salt solutions is concerned, and the experiments reported in this communication not only confirm Pel's observations but add positive evidence of resistance to immune serum; and they demonstrate, moreover, that the serum of the splenectomized animal presents no change in complementary activity and no distinct increase in antihemolytic property. This demonstration is conclusive, for in the hemolysis *in vivo* as well as *in vitro* three components are necessary; the corpuscles, the amboceptor, and the complement. If anticomplementary substances were present in the blood, they would be effective in diminishing the complementary action of the animal's serum. If the complement itself were small in amount or diminished in activity, it would be seen in the results of the test-tube experiments. The amboceptor is the constant and controllable factor in the experiment and the only uncontrollable agent in this connection is an anti-amboceptor or antihemolysin, which, from the results of our experiments, is not found in the three splenectomized animals, or in the animal which had been given immune serum, in greater amounts than in normal dogs. The remaining factor is the red blood corpuscle itself; and that it is in this cell that the increased resistance lies is shown by these experiments, which demonstrate beyond question that the corpuscles of the splenectomized animals and of the animals which had been given immune serum exhibit distinctly increased resistance to the definitely controlled action of specific amboceptor and complement.

Why the absence of the spleen increases the resistance of the red cells is still an open question, but the information obtained by this study, excluding as it does changes in the serum, narrows the problem to the red cells themselves. Of great significance are the results of the study of the blood of dog 43. This blood was used as a control on the supposition that an administration of a hemolytic serum might have caused the production of an antihemolysin. But this was not demonstrable either in this dog or in dog 24, which also had received hemolytic serum.

The conclusion is unavoidable that the increased resistance of the corpuscles, after the use of such a serum, is associated with the anemia caused by the hemolytic serum. This increased resistance of red cells in anemia is not uncommon, and, as has been stated, has been brought forth by Bottazzi, in connection with the action of pyrodin, as an explanation of the increased resistance of the red cells to this drug after splenectomy. That the increased resistance may be due to splenectomy alone and be entirely independent of the action of hemolytic substance introduced from without, is shown in the experiments with the blood of dogs 51 and 46, and has also been clearly demonstrated in the first and third communications of this series. One cannot therefore escape from the conclusion that the increased resistance of the red cells is a concomitant of the regeneration of the blood which follows the anemia caused by the removal of the spleen. That anemia does follow splenectomy has been shown in many investigations, and recently has been demonstrated anew in this laboratory by Musser;[18] that the return of the blood picture to normal is accompanied by increased resistance of the red cells to hemolytic sera has been shown in the third communication of this series, in which also it has been demonstrated that anemia has an important bearing on the production of hemolytic jaundice.

The main problem therefore is the explanation of the anemia following splenectomy. The possibility that this anemia may be due to an autohemolysin normally neutralized by the spleen, but operative in the absence of the spleen, is excluded by the control experiments here presented. There remains therefore the question of how the spleen regulates or controls or is otherwise concerned in the general process of blood destruction and regeneration, and to obtain information on this point, the changes in the lymph nodes and bone marrow, after splenectomy, are now receiving especial consideration.

CONCLUSIONS.

1. The erythrocytes of splenectomized dogs show increased resistance to the action of hypotonic salt solutions and to specific

[18] Musser, J. H., Jr., An Experimental Study of the Changes in the Blood Following Splenectomy, *Arch. Int. Med.*, 1912, ix, 592.

hemolytic immune serum. The degree of resistance appears to increase with the length of time that has elapsed after splenectomy.

2. This increased resistance of the erythrocytes is not due to an increased antihemolytic power of the animal's serum or to a diminished complementary value of the serum, but is a property depending upon the erythrocytes themselves.

3. Non-splenectomized animals receiving a single injection of specific hemolytic immune serum and developing a temporary anemia show likewise on recovery an increased resistance of the corpuscles without the presence of antihemolysin in demonstrable amount.

4. As anemia of varying grade is a characteristic result of splenectomy, it would appear that the increased resistance of the corpuscles is a concomitant of the regeneration of the red cells following such anemia and is thus analogous to the increased resistance of such cells not infrequently observed in various forms of experimental anemia.

5. There is no evidence to indicate that the anemia after splenectomy is due to the presence of hemolytic bodies, or that the increased resistance of the cells is due to antihemolytic bodies, accumulating in the serum as the result of the ablation of the spleen. It is evident therefore that the spleen in some way controls or regulates blood destruction (and regeneration ?), and in the hope of throwing light on the subject, an investigation of the bone marrow and lymph nodes of splenectomized dogs is now under way.

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

V. Changes in the Endothelial Cells of the Lymph Nodes and Liver in Splenectomized Animals Receiving Hemolytic Serum.*

By RICHARD M. PEARCE, M.D., and J. H. AUSTIN, M.D.

(*From the John Herr Musser Department of Research Medicine of the University of Pennsylvania, Philadelphia.*)

Plate 77.

In the course of our investigation[1] of the relation of the spleen to blood changes and to jaundice, routine histological examinations have been made. These have, for the most part, revealed only the well known lesions—liver necrosis, the degenerations occurring in epithelial organs, exudative lesions of the kidneys, and the general hemorrhages described by various workers[2] who have studied the

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, July 16, 1912.

[1] Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 363; Pearce, R. M., Austin, J. H., and Eisenbrey, A. B., II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals, *ibid.*, 375; Pearce, R. M., Austin, J. H., and Musser, J. H., Jr., III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice, *ibid.*, 758; Karsner, H. T., and Pearce, R. M., IV. A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood Corpuscles after Splenectomy, *ibid.*, 769.

[2] Kraus, R., Ueber Wirkungen der Hämolysine im Organismus, *Verhandl. d. deutsch. path. Gesellsch.*, 1903, v, 58.

Pearce, R. M., An Experimental Study of Nephrotoxins, *Univ. Penn. Med. Bull.*, 1903–04, xvi, 217; The Experimental Production of Liver Necrosis by Hemagglutinins, *Jour. Med. Research*, 1904, xii, 329; Concerning the Specificity of the Somatogenic Cytotoxins, *Jour. Med. Research*, 1904, xii, 1.

Fukuhara, Y., Zur Kenntnis der Wirkung der hämolytischen Gifte im Organismus, *Beitr. z. path. Anat. u. z. allg. Path.*, 1904, xxxv, 434.

Kraus, R., and Sternberg, C., Ueber Wirkungen der Hämolysine im Organismus, *Centralbl. f. Bakteriol., Orig.*, 1902, xxxii, 903.

action of hemolytic serum. The liver and lymph nodes, however, presented in a small group of animals changes which appear to be of significance in connection with the general problem of blood destruction in the absence of the spleen. The most striking of these changes, and the one that we shall describe, is the great increase in the phagocytic power of the endothelial cells of the liver and lymph nodes for red blood corpuscles.

It is not our intention to discuss the literature concerning the destruction of red cells. This has been well presented up to 1893 by Gabbi,[3] and up to 1901 by Hunter.[4] The more recent literature has added little either in fact or theory that is new. Out of the mass of contradictory statements there is uniformity of opinion on only two points: (1) that large endothelial cells of the spleen (the red blood corpuscle-carrying cells) have the power to engulf red blood cells; and (2) that the presence (in anemia and malaria) of blood pigment in the cells (Kupfer's cells) of the liver capillaries indicates that these cells play some part in the destruction of red blood cells. On the other hand, it is not generally admitted that the endothelial cells of the lymph nodes likewise have this power. That phagocytosis of red cells, wherever it occurs, leads ultimately to the freeing of hemoglobin which eventually reaches the liver and is transformed into bile pigment is the opinion of all who support the theory that this mechanism plays a part in the destruction of red blood cells. There is, however, no uniformity of opinion as to whether the hemoglobin is set free in the liver from red cells carried there by the phagocytes or whether it is set free by the phagocytes elsewhere and carried to the liver in another way.

It is only with one phase of the subject, the rôle of endothelial cells in engulfing red cells in the absence of the spleen, that we are concerned. Our hypothesis is that in the absence of the spleen the endothelial cells of the lymph nodes and liver compensate for the loss of similar cells of the spleen.

This possibility was first brought to our attention in the routine examination of tissues from splenectomized dogs which had received specific hemolytic immune serum. So striking were some of

[3] Gabbi, D., Ueber die normale Hämatolyse mit besonderer Berücksichtigung der Hämatolyse in der Milz, *Beitr. z. path. Anat. u. z. allg. Path.*, 1893, xiv, 351.

[4] Hunter, W., Pernicious Anemia, London, 1901.

the pictures, that we undertook, for the sake of control, the study of the liver and lymph nodes from a number of normal dogs, of normal dogs receiving hemolytic serum, and of dogs which had been splenectomized for various lengths of time, but which had not received hemolytic serum.

Lymph Nodes.—The lymph nodes studied have been, for the most part, the mesenteric, gastrohepatic, prevertebral, and bronchial. In the normal animal these have been examined more particularly for the frequence of mitosis, for the number of endothelial cells in the sinuses, and for the presence of cells containing red blood corpuscles. Careful study of nodes from five normal animals showed that mitotic figures are found only after prolonged search and are usually limited to the follicles. The number of endothelial cells varies, but usually is not great, and they never occur in large masses in the sinuses. These cells, however, not infrequently contain old blood pigment, and occasionally a cell may be seen containing one or two red blood corpuscles.

The lymph nodes of five animals splenectomized for 3, 4 (2), 39, and 84 days and not subjected to the action of hemolytic serum have been studied in the same way. In two animals representing respectively 4 and 84 days, the lymph nodes differed in no way from the normal; in the other animals mitotic figures were abundant in the follicles and the endothelial cells in the sinuses were greatly increased in number. Prolonged search, however, failed to demonstrate mitotic figures in the latter cells, and although they occasionally contained one or two red blood corpuscles, this power of phagocytosis did not appear to be greater than in the non-splenectomized animal. The increase in number of endothelial cells was, however, very striking.

The literature of splenectomy offers little aid in determining the histological changes occurring in the lymph nodes after removal of the spleen. In the literature at our disposal no definite descriptions have been found except those of Warthin,[5] who found in

[5] Warthin, A. S., The Changes Produced in the Hemolymph Glands of the Sheep and Goat by Splenectomy, Contributions to Medical Research, Dedicated to Victor Clarence Vaughan, Ann Arbor, 1903, 216.

sheep and goats an increase in the phagocytic power of the endothelial cells for red blood corpuscles.[6] Gabbi,[7] who worked with the guinea pig, states that a transient increase of the red blood corpuscle-carrying cells may possibly occur in early periods after splenectomy, but that after three to six months they are no more abundant than in the normal animal.

The lymph nodes of five normal dogs which had received specific hemolytic immune serum and had died or been chloroformed after periods varying from twenty-four hours to nine days, showed the lesions usually described as common to various cytotoxic sera and especially to lymphotoxic sera.[8] These are edema, increase of polymorphonuclear leucocytes, focal areas of necrosis, abundant mitotic figures in the follicles, and slightly greater frequence of large endothelial cells capable of phagocytosis of red cells.

Of animals that had been splenectomized and had received hemolytic serum as well, twelve were available for histological examination. Of these, three showed no change in the lymph nodes and five showed a well marked increase in the number of endothelial cells in the sinuses, but no increase in power of phagocytosis. In the four remaining animals the sinuses contained a great number of large endothelial cells filled with red blood corpuscles. The analysis of these findings is somewhat difficult, as three factors must be considered: (1) the length of time after splenectomy; (2) the lapse of time between administration of hemolytic serum and the death of the animal; and (3) the degree of red cell destruction caused by the serum.

These factors are brought out in the following table.

[6] Warthin also describes a new formation of hemolymph nodes. This we have not observed in any of the nineteen splenectomized dogs. This, however, is not surprising as the demonstration of hemolymph nodes in the dog is difficult, and, moreover, only three of our animals have survived splenectomy for more than four months.

[7] Gabbi, D., *loc. cit.*

[8] Flexner, S., The Pathology of Lymphotoxic and Myelotoxic Intoxication, *Univ. Penn. Med. Bull.*, 1902, xv, 287.

Bunting, C. H., The Effects of Lymphotoxins and Myelotoxins on the Leucocytes of the Blood and on the Blood-Forming Organs, *Univ. Penn. Med. Bull.*, 1903–04, xvi, 200.

Experiment No.	Period of splenectomy.	Period after serum.	Effect of serum.	Histology.
I	3 dys.	36 hrs.	Hemoglobinuria	Extreme phagocytosis of red cells.
II	10 mos.	36 hrs.	Severe jaundice	Well marked phagocytosis of red cells.
III	$9\frac{1}{2}$ mos.	18 hrs.	No record	Well marked phagocytosis of red cells.
IV	$7\frac{1}{2}$ mos.	48 hrs.	Hemoglobinuria	Moderate phagocytosis of red cells.
V	15 dys.	3 dys.	Hemoglobinuria	Negative.
VI	27 dys.	3 dys.	Hemoglobinuria	Proliferation of endothelial cells.
VII	33 dys.	4 dys.	Jaundice	Proliferation of endothelial cells.
VIII	65 dys.	8 dys.	Hemoglobinuria	Proliferation of endothelial cells.
IX	6 dys.	9 dys.	Hemoglobinuria	Negative.
X	3 dys.	9 dys.	No hemoglobinuria or jaundice	Negative.
XI	103 dys.	10 dys.	No hemoglobinuria (spontaneous jaundice)	Proliferation of endothelial cells.
XII	25 dys.	15 dys.	Jaundice	Proliferation of endothelial cells.

From this analysis it is seen that the proliferation of the endothelial cells did not occur in the animals (V, IX, and X) splenectomized for periods of from three to fifteen days, but was evident in five (VI, VII, VIII, XI, and XII) in which the time elapsing since splenectomy was 27 to 103 days. On the other hand, the lymph nodes of these animals did not present evidence of increased phagocytosis of red cells. Whether this was due to the period which had elapsed (three to fifteen days) since injection of serum cannot be determined, but this was probably the case. Certainly it was not due to failure of hemolysis, for at least two of these animals (VI and VIII) presented evidence of extreme blood destruction. That the period of time elapsing may be an important factor is shown by the fact that all animals (four) presenting evidence of extensive phagocytosis of red cells represent periods of eighteen to forty-eight hours after injection of the serum. In the absence of exact knowledge of the length of time necessary for the destruction of red cells by phagocytic endothelial cells, it is useless to offer surmises, but one cannot escape the fact that in this investigation all evidence of active phagocytosis is seen in animals dying within forty-eight hours. It is possible therefore that the destruction of red cells by phagocytosis may be completed within forty-eight hours and this view is supported by the frequence with which pigment is found in the lymph nodes at later periods.

It is also evident that the time elapsing since splenectomy bears no relation to the occurrence of phagocytosis of red cells, for the most marked example of the latter was seen in a dog dying three days after splenectomy, while moderate and well marked phagocytosis occurred likewise after 7½, 9½. and 10 months.

Liver.—Examination of the stellate endothelial cells (Kupfer's cells) of the liver has been rendered difficult on account of the intense congestion and abundant necroses which occur after the administration of hemolytic immune serum. For this reason we have not always been able to correlate the evidence of phagocytosis in the liver with the lesion described in the lymph nodes during the early (forty-eight hour) period. Definite evidence, however, of phagocytosis has been found in four animals, representing periods of 1, 2, 8, and 9 days after the administration of serum, and representing, respectively, periods of 3 days, 7½ months, 65 days, and 6 days after splenectomy. Also in a fifth animal, twenty-five days after splenectomy, and fifteen days after the administration of the serum, the cells of the capillaries contained small balls of yellow pigment, apparently representing altered hemoglobin.

As controls we have examined the livers of several normal dogs and of nine splenectomized dogs not receiving serum, but without finding evidence of phagocytosis on the part of the cells of the liver capillaries, or of proliferation of these cells. The splenectomies in this series represented periods of from three to eighty-four days, five under ten days and three over twenty days.

Likewise we have examined the livers of nine normal dogs receiving hemolytic serum. In two of these the endothelial cells appeared to be increased somewhat in number, and no undoubted evidence of phagocytosis could be obtained. All other livers examined showed no changes in the cells of the capillaries.

The details of some of these observations follow:

PHAGOCYTOSIS OF RED BLOOD CELLS BY STELLATE CELLS OF THE LIVER.

Dog 32.—Splenectomy was performed under ether anesthesia on July 19, 1911; spontaneous jaundice was noted on March 8, 1912, and specific hemolytic immune serum injected intravenously on this date. Hemolysis resulted. The red cells dropped within three hours from 6,120,000 to 5,200,000 per cubic millimeter, and the hemoglobin, after twenty hours, to 42 per cent. Death occurred after forty-eight hours.

Histology.—The liver cells are pale, granular, stain poorly, and present here and there small areas of focal necrosis. The capillaries are dilated and contain much granular material and, as seen by the low power, numerous isolated round and oval clumps of red blood corpuscles. By higher power of the microscope these clumps of red cells are found to be, in large part, within endothelial cells (figure 4). Some of the red cells stain well with eosin, others appear as shadows. Other endothelial cells are seen which contain mere fragments of red cells or masses of granular, yellow pigment, or large, yellow hyaline balls of apparently fused, red cells. Attempts to demonstrate similar phagocytic cells in the large vessels of the liver and of other organs failed; they were present, however, in the sinuses of the lymph nodes.

PHAGOCYTOSIS OF RED CELLS BY ENDOTHELIAL CELLS OF THE LYMPH NODES.

Dog 34.—Splenectomy was performed under ether anesthesia on March 11, and hemolytic serum administered intravenously on March 14. Death occurred on March 15 after reduction of red cells to 1,960,000 and hemoglobin to 57 per cent. Hemoglobinuria was marked.

Histology.—A mesenteric lymph node shows hemorrhage, edema, and extensive infiltration with polymorphonuclear leucocytes. The sinuses, both peripheral and central, are closely packed with large, pale, endothelial cells, nearly all of which contain red blood cells, a single high power field showing thirty to forty phagocytic cells (figures 1 and 2). The number of engulfed red cells varies but is usually large, ten to twenty not infrequently being found in a single cell. In many endothelial cells, on the other hand, the red cells have fused to form large, round, or oval hyaline masses still staining deeply with eosin. Between the phagocytic cells is much granular, eosin-staining material suggesting disintegrated red cells, mingled with serum, through which run irregular threads of fibrin. Here and there in the follicles are small areas of necrosis. Moderate leucocytic infiltration is seen throughout the section. Phagocytic cells cannot be demonstrated in the blood-vessels or in a tangle of lymphatic vessels present at one side of the node.

Other lymph nodes (gastrohepatic, prevertebral, and bronchial) present the same lesions.

The liver of this animal showed widespread necrosis but in the non-necrotic areas phagocytic endothelial cells are found in the capillaries (figure 3).

SUMMARY.

In a large proportion of dogs that have been splenectomized for periods of two weeks or more, one finds a great increase in the number of endothelial cells of the lymph nodes. In most splenectomized dogs that succumb to an injection of hemolytic immune serum within forty-eight hours, the sinuses of the lymph nodes contain large numbers of endothelial cells, phagocytic for red cells. This is not seen in normal dogs receiving hemolytic serum. Like-

wise a similar power of phagocytosis is seen frequently in the stellate cells of the capillaries of the liver. Both in the lymph nodes and the liver these cells appear to be formed *in situ;* we find no evidence that they have been transported to these organs.

Such findings suggest the development of a compensatory function on the part of the lymph nodes and possibly of the liver. Normally the spleen contains cells which have the power to engulf and presumably to destroy the red blood corpuscles. In certain pathological conditions this function is frequently greatly augmented and may sometimes be shared by the lymph nodes, for example, in typhoid fever, as was first clearly shown by Mallory.[9] Our observations suggest that in the absence of the spleen, this function of forming red blood corpuscle-phagocyting cells, normally a minor activity of the lymph nodes, becomes highly developed in the latter organs, and that these cells, and the stellate cells of the liver, thus assume, in part at least, the function of destroying red blood corpuscles by phagocytosis.

In view of the somewhat limited material at our disposal, we offer this, not as definitely conclusive, but as evidence which, in connection with the work of others, is highly suggestive of the possibility of the lymph nodes assuming some of the function of the spleen.

Whether this activity of the endothelial cells of the lymph nodes and the liver has any bearing on the anemia that follows splenectomy and on the occurrence of spontaneous jaundice in the late periods after splenectomy, is not yet clear.

[9] Mallory, F. B., A Histological Study of Typhoid Fever, *Jour. Exper. Med.*, 1898, iii, 611.

EXPLANATION OF PLATE 77.

The drawings were made with the camera lucida and with a Spencer microscope, objective, 4 mm., ocular, 8.

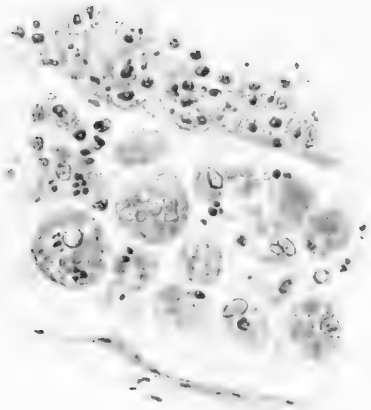
Figures 1, 2, and 3 represent lesions in a dog that was splenectomized on March 11, 1912, received hemolytic serum on March 14, and died on March 15. The actual lapse of time was about thirty-six hours.

FIG. 1. Peripheral sinus of a mesenteric lymph node containing large numbers of endothelial cells filled with red blood corpuscles and occasionally also polymorphonuclear leucocytes.

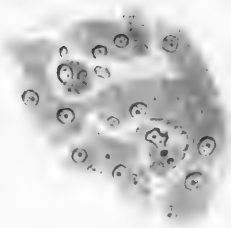
FIG. 2. Similar cells in a central sinus of the same lymph node.

FIG. 3. A section of liver with two Kupfer cells containing red blood corpuscles.

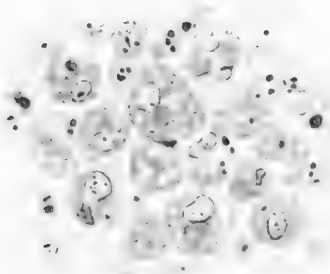
FIG. 4. Similar to figure 3, but from a dog that was splenectomized on July 19, 1911, received hemolytic serum on March 8, 1912, and died after forty-eight hours.



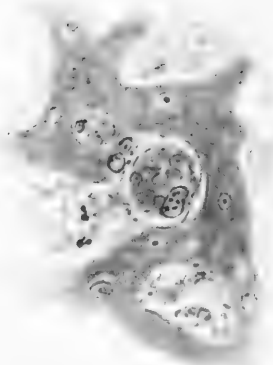
1



3



2



4

NITROGEN RETENTION IN THE BLOOD IN EXPERIMENTAL ACUTE NEPHRITIS OF THE CAT.*

By OTTO FOLIN, PH.D., HOWARD T. KARSNER, M.D., AND W. DENIS, PH.D.

(From the Laboratory of Pathology (Phillips Fund) and the Laboratory of Biological Chemistry of the Harvard Medical School, Boston.)

The literature and problems of experimental nephritis have been recently considered by Pearce[1] and it is therefore unnecessary to repeat them at this time. Considerable work has been done on the subject of nitrogen metabolism in acute nephritis and somewhat variable results have been attained. The development of the colorimetric methods for nitrogen determinations in the blood[2] has opened up new fields for work, and the object of the present study is to discover from the standpoint of blood analysis the possible retention of nitrogen in experimentally produced nephritis.

The types of nephritis selected for the study are those produced by uranium nitrate, by potassium chromate, and by cantharidin. This study must therefore be looked upon as preliminary to a more extensive series of other types of acute nephritis. A brief description of the type of nephritis produced in the cat by the poisons mentioned precedes each group of experiments; a more extensive study of the anatomical lesions found in the cat will be made the subject of a separate report.

The cats were kept under observation for from a week to ten days preceding the experiment. The animals were fed at 9 A. M. and bled at 3 P. M. The diet, a meat diet, was regulated so as to keep the weight constant, and frequent examinations of the urine were made in order to be sure that the animal had normal kidneys. All injections of the poisons were subcutaneous and the blood

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, July 31, 1912.

[1] Pearce, R. M., The Problems of Experimental Nephritis, Harvey Lectures, 1909–10, Philadelphia and London, 1910, 17; also in *Arch. Int. Med.*, 1910, v, 133.

[2] Folin, O., and Denis, W., Protein Metabolism from the Standpoint of Blood and Tissue Analysis, *Jour. Biol. Chem.*, 1912, xi, 164; New Methods for the Determination of Total Non-Protein Nitrogen, Urea and Ammonia in Blood, *Jour. Biol. Chem.*, 1912, xi, 527.

was obtained by aseptic puncture of the heart. Heart puncture was found to be satisfactory unless an extremely large needle was used. The blood was measured in volumetric pipettes. Five c.c. were withdrawn from the larger cats, 2 c.c. from the smaller animals, the amount being constant throughout each experiment. Further details of the technique are described in the second paper of Folin and Denis.[3]

Albuminuria was routinely tested for both by the heat and acid and by the nitric acid contact methods. The examination for casts was made in centrifuged specimens of urine, but frequently was unsatisfactory because of the large amounts of fat in the urine and the rich crystalline sediment.

URANIUM NEPHRITIS.

The most marked change histologically is in the epithelium, where cloudy swelling is found to be very extensive, in the later stages going on to complete necrosis. The glomerular tufts show moderate intercapillary proliferation which in later stages is obscured by the pushing back into the subcapsular space of epithelium of the type found in the proximal convoluted tubules.

Four cats were used as follows:

Cat 1.—Weight 3,550 gm. Given 0.002 gm. of uranium nitrate subcutaneously. Bled on the 3d day. Killed on the 3d day. Slight albuminuria on the 2d and 3d days. Few granular casts.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	32[4]	20
48 hrs. after injection.	41	21

Cat 2.—Weight 1,700 gm. Given 0.0008 gm. of uranium nitrate subcutaneously. Bled on the 3d, 5th, 7th, and 9th days. Killed on the 9th day. Marked albuminuria up to the 6th day, then slight until termination. Few granular casts in earlier days.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	37	20
48 hrs. after injection.	50	27
96 hrs. after injection.	55	32
168 hrs. after injection.	44	19
216 hrs. after injection.	44	20

Cat 3.—Weight 4,000 gm. Given 0.002 gm. of uranium nitrate subcutaneously. Bled on the 2d, 6th, and 8th days. Found dead in cage on the 9th day (hemopericardium). Marked albuminuria in the earlier days remaining considerable to the end of the experiment.

[3] Folin, O., and Denis, W., *loc. cit.*

[4] The figures represent milligrams of nitrogen in 100 gm. of blood.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	31	21
72 hrs. after injection.	46	26
168 hrs. after injection.	100	36
216 hrs. after injection.	82	38

Cat 4.—Weight 1,540 gm. Given 0.0008 gm. of uranium nitrate subcutaneously. Bled on the 4th, 6th, 8th, and 10th days. Allowed to live. Albuminuria marked until the 8th day, very slight thereafter, disappearing on the 14th day. Hyaline and granular casts persisted until the 20th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	37	22
72 hrs. after injection.	55	30
144 hrs. after injection.	50	25
192 hrs. after injection.	42	20
240 hrs. after injection.	37	22

The doses of uranium nitrate employed were sufficient to produce a well marked nephritis anatomically, most severe in the case of cat 3. This cat also showed the most marked albuminuria and the highest nitrogen content in the blood after injection. The dose of uranium was proportionately very little larger than that given cat 1, but the process evidently was more severe both anatomically and functionally. It can fairly be said that the lesion produced by the injection of uranium nitrate results in the accumulation of non-protein and urea nitrogen in the blood much in excess of the normal amount, and as far as these experiments go, roughly in accordance with the degree of albuminuria and anatomical lesions. In all cases the retention appeared within 24 hours, persisted for as long as 216 hours, and in one case returned to normal after 240 hours.

CHROMATE NEPHRITIS.

The kidney lesion is almost purely tubular in type, the epithelium showing marked cloudy swelling and the appearance of hyaline droplets, the latter particularly in the distal convoluted tubules. The glomeruli occasionally show slight leucocytic infiltration or even early intercapillary proliferation, but for the most part are entirely free of involvement other than congestion.

Cat 5.—Weight 5,450 gm. Given 0.010 gm. of potassium chromate subcutaneously. Bled on the 2d and 4th days. Albuminuria slight from the 2d day to

the end of the experiment. Hyaline, granular, and fatty casts on the 2d day. Killed on the 4th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	40	19
24 hrs. after injection.	48	23
72 hrs. after injection.	38	24

Cat 6.—Weight 3,980 gm. Given 0.015 gm. of potassium chromate subcutaneously. Bled on the 2d, 4th, 6th, 8th, and 10th days. Albuminuria on the 2d day becoming progressively worse. Refused to eat after the 8th day. No casts at any time, but much epithelium and granular material in sediment. Killed on the 10th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection (1 week).	58	34
24 hrs. after injection.	40	22
72 hrs. after injection.	56	38
120 hrs. after injection.	60	40
168 hrs. after injection.	68	44
216 hrs. after injection.	44	30

Cat 7.—Weight 2,250 gm. Given 0.010 gm. of potassium chromate subcutaneously. Bled on the 3d, 5th, and 7th days. After bleeding on the 7th day, a second dose of 0.020 gm. of potassium chromate was given, and bleedings were continued on the 9th, 11th, and 14th days. Albuminuria did not appear until the 3d day and was slight until after the second chromate injection, becoming marked on the 8th, 9th, 10th, and 11th days, but disappearing on the 14th day. Casts did not appear until after the second injection, a few granular casts being found on the 8th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	40	28
48 hrs. after injection.	45	23
96 hrs. after injection.	46	34
144 hrs. after injection.	50	36
192 hrs. after injection.	58	38
216 hrs. after injection.	50	36
312 hrs. after injection.	54	34

Cat 8.—Weight 1,620 gm. Given 0.015 gm. of potassium chromate subcutaneously. Bled on the 2d day. Slight albuminuria on the 2d day. Killed on the 2d day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	56	36
24 hrs. after injection.	55	35

The accumulation of nitrogen in the cats injected with potassium chromate is obviously less marked than in the uranium animals.

In cats 6 and 7 the accumulation appeared to be progressive, reaching its height several days after injection. In the case of cat 7 a second injection during the course of the disease appeared to increase the blood nitrogen to a considerable degree, more than would have been expected in the natural course of events if one might judge from cat 6. The degree of retention is apparently not at all proportional to the dose of drug employed but is roughly in accordance with the degree of albuminuria. The return to normal appeared in a severe case (cat 6) after 216 hours, and in a less severe case after 74 hours.

CANTHARIDIN NEPHRITIS.

Both glomeruli and epithelium show extensive lesions. The glomeruli show well marked intercapillary and intracapillary proliferation and moderate leucocytic infiltration. The lesion of the glomerular tuft is more marked than in either of the other forms studied. The tubular epithelium shows well marked cloudy swelling and the formation of numerous hyaline granules in the cells,—a change almost identical with that seen in chromate nephritis. Histologically the cantharidin lesion represents the chromate lesion with a superadded glomerular lesion of considerable severity.

Cat 9.—Weight 3,170 gm. Given 0.001 gm. of cantharidin in acetic ether subcutaneously. Bled 6 hours after injection and again on the 6th day. Killed on the 6th day. No albuminuria at 6 hours, moderate albuminuria at 48 hours and to the end of the experiment. No casts.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	36	24
6 hrs. after injection.	36	24
126 hrs. after injection.	40	28

Cat 10.—Weight 3,040 gm. Given 0.001 gm. of cantharidin in acetic ether subcutaneously. Bled 12 hours after injection and again on the 6th day. Slight albuminuria at 12 hours, disappearing before the 6th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	36	24
12 hrs. after injection.	45	32
132 hrs. after injection.	40	28

Cat 11.—Weight 3,700 gm. Given 0.001 gm. of cantharidin in acetic ether subcutaneously. Bled on the 3d day and then killed. Slight albuminuria.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	36	24
48 hrs. after injection.	41	26

Cat 12.—Weight 2,870 gm. Given 0.001 gm. of cantharidin in acetic ether subcutaneously. Bled on the 2d, 4th, and 7th days. Killed on the 7th day. Albuminuria on the 2d day, disappearing before the end of the experiment.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	36	22
24 hrs. after injection.	45	33
72 hrs. after injection.	41	26
144 hrs. after injection.	42	26

Cat 13.—Weight 2,760 gm. Given 0.002 gm. of cantharidin in acetic ether subcutaneously. Bled on the 2d, 4th, and 6th days. Vomited on the 1st day and ate poorly throughout the experiment. Albuminuria marked to end of the experiment. Killed on the 6th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	36	20
24 hrs. after injection.	66	41
72 hrs. after injection.	44	23
120 hrs. after injection.	40	29

Cat 14.—Weight 2,700 gm. Given 0.001 gm. of cantharidin in acetic ether subcutaneously. Bled on the 3d, 5th, and 7th days. Vomited on the 1st day and ate poorly up to the 5th day. Marked albuminuria throughout the experiment. Killed on the 7th day.

Time of bleeding.	Non-protein nitrogen in mg.	Urea nitrogen in mg.
Before injection.	46	28
48 hrs. after injection.	64	38
96 hrs. after injection.	44	26
144 hrs. after injection.	50	34

In the nephritis produced by cantharidin the nitrogen retention is marked in the more severe cases. Of all the animals used, those receiving cantharidin were the only ones showing gastro-intestinal disturbance. Vomiting frequently appeared within a few hours after the injection, and anorexia persisted in some cases for four or five days.

The accumulation of blood nitrogen is clearly evident in all the cases; it shows no dependence on the amount of cantharidin injected, but accords with the degree of general disturbance and albuminuria; there was no discernible ratio between the degree of

anatomical involvement and the nitrogen accumulation. The increased nitrogen content, not apparent at six hours, became clearly evident at twelve hours, was most marked at twenty-four hours, and tended gradually to subside.

SUMMARY.

It will be seen that uranium nephritis, which involves both tubules and glomeruli, the former more markedly than the latter, produces a marked accumulation of nitrogen in the blood. Chromate nephritis, which involves almost exclusively the tubules, produces only moderate retention of nitrogen. Cantharidin nephritis which involves both tubules and glomeruli, the latter more severely than does uranium, produces a marked accumulation of nitrogen, beginning early and persisting for a considerable period. The experiments were controlled by testing the blood of normal cats kept under the same conditions, these animals showing only slight variations from day to day. This general statement is in accordance with the physiological classification of these nephritides except that the retention in uranium occurs at an early stage, where, according to the physiological studies of Schlayer and his associates,[5] and of Pearce, Hill, and Eisenbrey,[6] the vascular changes have not as yet appeared. It must be noticed that in the three types of nephritis that form the subject of this investigation, anatomical study shows the glomerulus to be distinctly involved in the two forms where accumulation of nitrogen in the blood is most marked, a condition indicating that although almost pure tubular involvement produces only moderate accumulation, the additional involvement of the glomerulus is extremely important in leading to a retention of nitrogenous waste products.

[5] Schlayer and Hedinger, Experimentelle Studien über toxische Nephritis, *Deutsch. Arch. f. klin. Med.*, 1907, xc, 1; Takayasu, R., Ueber die Beziehungen zwischen anatomischen Glomerulusveränderungen und Nierenfunktion bei experimentellen Nephritiden, *idem*, 1907, xcii, 127; Schlayer and Takayasu, R., Untersuchungen über die Funktion kranker Nieren, *München. med. Wchnschr.*, 1909, lvi, 2201.

[6] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., Experimental Acute Nephritis: The Vascular Reactions and the Elimination of Nitrogen, *Jour. Exper. Med.*, 1910, xii, 196.

The accumulation of non-protein nitrogen in the blood and tissues is not large when compared with the total intake or elimination of nitrogen, and consequently it is practically impossible by means of ordinary nitrogen equilibrium experiments to demonstrate the fact of the retention, to say nothing of determining the degree of accumulation of waste products accompanying nephritis. That both can be demonstrated by the method employed in this research is clearly shown by the figures recorded above.

A CONTRIBUTION TO THE PATHOLOGY OF PRIMARY SPLENOMEGALY (GAUCHER TYPE), WITH THE REPORT OF AN AUTOPSY ON A MALE CHILD FOUR AND ONE HALF YEARS OF AGE.*

By F. S. MANDLEBAUM, M.D.

(*From the Pathological Laboratory of Mount Sinai Hospital, New York.*)

PLATES 78–84.

Since the publication of the first case of this disease by Gaucher (1) in 1882, only nine additional instances have been recorded in which the diagnosis was made by histological examination. The eleventh case now to be reported has the distinction of being the youngest recorded case in which an autopsy was performed.

No cases were reported from 1882 until 1895, when Collier (2) published the second case. In the same year Picou (3) reported a case before the Anatomical Society of Paris, and a year later, in association with Ramond (4), the case was published in full. The first case in this country was described by Bovaird (5) in 1900. In 1904, in collaboration with Brill and Libman (6), it was my privilege to demonstrate the specimens of two cases, the first of which was published the following year (7).

The earliest case in German literature was reported by Schlagenhaufer (8), who demonstrated the specimens in 1906 and published his case in detail the following year (9). The seventh case, briefly described in 1907 by von Herczel (10), had been operated upon two years before for removal of the spleen, but the true nature of the process was not recognized at the time. In the same year Marchand (11) reported the findings in his case, and the specimens were demonstrated at a meeting of the German Pathological Society by Risel (12) in 1909. In association with Brill and Libman (13) in 1909 I had the opportunity of publishing the findings in our second case, and in the same year, after having completed a thorough study of Marchand's case, Risel (14) reported the full result of his work in an elaborate and most comprehensive paper.

* Received for publication, July 20, 1912.

The tenth case was published by de Jong and Van Heukelom (15) in 1910.

These are the only cases in the literature in which the diagnosis was established anatomically, either by examination of the spleen or by autopsy. Several of the authors just mentioned have noted splenic enlargement in one or more members of the same families and have included them in their tabulated cases of this disease. Inasmuch as the Gaucher type of splenomegaly has been proved to be a family form of disease, it is more than reasonable to assume that their observations and diagnoses were correct, but for scientific statistical purposes it is thought inadvisable to include these cases. It is interesting to note, however, that the addition of such cases would bring the total number up to twenty.

In this connection it may be stated that the diagnosis was made during life and subsequently confirmed in only four of the authentic cases. To Brill (16) belongs the credit of isolating these cases from the large group of splenic hypertrophies and establishing the diagnosis on a clinical basis. For in 1901, Brill, who had observed and treated two cases for sixteen years, and up to this time had considered them unique, says: "From what has been adduced it may be held that my cases correspond only with those described by Gaucher, Picou and Ramond, Collier, Weichselbaum (even doubtful), and Bovaird." The diagnosis in both of these cases was confirmed at autopsy three years later.[1] The diagnosis in de Jong and Van Heukelom's case, in the family of which two other members were affected, was also confirmed. In the case that serves as the basis for this paper, the diagnosis was made during life by Dr. M. S. Reuben (17). One other child was affected in this family. In the eleven authentic cases on record nine occurred in females and two in males. The male cases are Brill's first case and the present one.

Before proceeding to the history and autopsy findings in this case, it seems advisable to sketch very briefly the interpretations and conclusions made by previous writers. Lack of space prevents any reference to the histories or microscopic examinations.

[1] In this family there were four cases in one generation.

HISTORICAL.[2]

1. Gaucher decided that his case was a peculiar idiopathic hypertrophy of the spleen, and on account of the histological character he regarded it as a primary epithelioma of that organ.

2. Collier described his case as an endothelioma of the spleen. The Morbid Growths Committee of the London Pathological Society to whom he submitted his slides called attention to the presence of an enormous number of endothelial-like cells, but decided against its sarcomatous or lymphadenomatous nature. In conclusion the Committee stated that: "This appearance is similar to that which is not uncommon in the lymphatic glands, and was present in the lymphatic glands in the present case."[3]

3. Picou and Ramond declared that the case was a primary epithelioma of the spleen beginning as a benign process, finally becoming malignant and involving the lymph nodes. Cornil, who discussed the case when first reported by Picou, called attention to the similarity of the large cells to those frequently seen in glandular hyperplasia, and to the distinct connective tissue network between the cells. In his opinion the process was a primary hypertrophy of the spleen with proliferation of the reticulum.

4. Bovaird considered the process to be "a hyperplasia of the spleen characterized by an unusual development of endothelial cells and the transformation of a considerable part of the organ into dense connective tissue." The same process affects the lymph nodes and the liver. It is confined in the latter organ to the lymph spaces in the perilobular connective tissue. The author also suggests "the action of some systemic poison affecting several members of a family."[4]

5. Brill, Mandlebaum, and Libman stated that the large cells arose "directly from the endothelial lining of the pulp spaces, or, more properly, venous capillaries" of the spleen. The presence of the large cells in the bone marrow was noted in their case for the first time.[5]

6. Schlagenhaufer concluded that the process was a systemic disease of the lymphatic-hemapoietic apparatus, with proliferation of the reticular structures. As tuberculosis was present in his case he decided that some toxin, probably of a tuberculous nature but without other manifestations of true tuberculosis, was the most probable etiological factor.[6]

7. von Herczel thought that the spleen was the seat of an inflammatory process or of a benign new growth, and was inclined to the latter opinion. The microscopic examination was made by Krompecher, who stated that the lesion was a complicated one and very difficult to interpret. The slides were subsequently submitted to Risel, who made the correct diagnosis.

[2] The cases will be reviewed in the order in which they appeared in the literature.

[3] A sister died at the age of eight years, and the spleen weighed twenty-five ounces. No microscopic examination was made.

[4] A sister was under observation with an enlarged abdomen and an enormous hypertrophy of the spleen and liver. She was still alive at the date of publication.

[5] This case was under treatment for nineteen years, and is the first male case published.

[6] A marked splenic hypertrophy was present in two sisters of this patient.

8. Marchand was of the opinion that the process is not an ordinary protoplasmic hyperplasia or a tumor, but a deposit of some foreign substance, which, after the action of certain chemicals, leaves a vacuolated network in the cells. Inasmuch as he occasionally saw cells with fibrils, he finally concluded that the cells might have their origin from either reticulum or endothelium.

9. Brill, Mandlebaum, and Libman stated that the spleen, liver, lymph nodes, and bone marrow "show the presence of large cells with small nuclei and a peculiar hyaline cytoplasm, which arise from the endothelium or normal reticulum, and the presence of pigment containing iron. The etiology is unknown, though a peculiar susceptibility of the endothelium or reticulum of the hemapoietic apparatus to some unknown toxic agent is most likely present." They also stated that when tuberculosis was found in these cases, it must be considered as a superimposed process.

8. Risel, in discussing Marchand's case, considered that the large cells are not derived from endothelium but probably arise in the reticulum of the lymphatic-hemapoietic system, the lymph adenoid tissue being unaffected. In the lymph nodes, however, the cells may arise from the endothelium of the lymph sinuses late in the course of the disease, as well as from the intrafollicular reticulum. The presence of cells in the connective tissue spaces of Glisson's capsule is difficult to explain unless one places the liver in the lymphatic-hemapoietic system, by virtue of its embryologic importance in the process of blood formation. The presence of cells in the venous sinuses of the spleen is difficult to understand if they arise in the reticulum, but the author shows how the lining membrane of the sinus may be injured by a swelling of the organ, thus allowing the cells to enter. He states that he never saw positive evidence of direct transition of reticulum into cells, but the impression was made that such must be the case.

10. De Jong and Van Heukelom decided that the cells arise from the reticulum, but stated that the possibility of an endothelial origin could not be denied. The liver continued to enlarge after a splenectomy, and they attribute this to a proliferation of the cells previously carried there from the spleen. Their case recovered from the operation, and they had no opportunity to examine the liver, nodes, or marrow.[7]

The history of the present case is as follows:

The patient, a boy aged 4½ years, was admitted to Mount Sinai Hospital on February 26, 1912, to the service of Dr. Lilienthal.[8]

The father and mother are both healthy; no history of tuberculosis or syphilis in the family. There were four children in all; one sister of eleven years is alive and well, and one sister died three years ago, at the age of eight, having had a slow but progressive enlargement of the abdomen for four years. Her liver extended 10 cm. below the free border. The spleen was felt beyond the median line, filling the left iliac cavity. There was no history of hemorrhage or skin pigmentation. Blood examination: 3,440,000 red cells; 6,200 leucocytes;

[7] Hypertrophy of spleen and liver were noted in a brother and sister.

[8] I wish to thank Dr. Lilienthal for his kindness in placing the clinical data at my disposal.

hemoglobin 68 per cent.; differential count normal. This child suddenly developed dyspnea and cyanosis, and died. No autopsy was performed. A second sister, aged nine years, has an enlarged liver which can be felt two inches below the free border, but as the spleen is not enlarged, the diagnosis is still somewhat obscure.

The little boy's early history was uneventful, except for measles a year ago. Shortly afterwards he was thrown down by a horse, and the ambulance surgeon told his mother that the child had an enlarged spleen and liver. Several physicians were consulted, and finally he was brought to the service of Dr. Herrman at the Vanderbilt Clinic. Examination at this time by Dr. Reuben showed a distinct discoloration of the face, from a light yellow to a golden hue, most marked at the bridge of the nose and around the eyes; conjunctivæ not discolored. The liver was palpable at the umbilicus; the spleen extended two inches below the umbilicus and over to the right mammary line. Superficial lymph nodes not enlarged; no bone tenderness; Wassermann reaction negative. Blood examination: 2,208,000 red cells; 500 leucocytes; hemoglobin, 35 per cent.; differential count normal; no abnormal cells. Dr. Reuben considered this to be a case of primary splenomegaly of the Gaucher type. He published his observations at the time, and referred the case to the hospital for splenectomy.

On admission to the hospital the only complaint was slight pain in the right hypochondriac region, and a history of occasional epistaxis. The physical examination corresponded to that given above. The urine contained phosphates; specific gravity, 1,030. Temperature, 99° F.; respirations, 22; pulse, 104. Blood examination at this time: 4,000,000 red cells; 4,200 leucocytes; hemoglobin, 68 per cent.; color index, 0.85. Differential count: polynuclears, 70; large lymphocytes, 25; small lymphocytes, 3; mononuclears, 1; myelocytes, 1; no normoblasts; slight poikilocytosis and anisocytosis.

Splenectomy was performed on March 1, 1912, by Dr. Wiener. The spleen extended downwards to the floor of the pelvis; extensive adhesions were found, the upper pole being attached to the stomach; several large veins near the tail of the pancreas were ligated. The entire operation was completed in one half hour, the child losing less than one ounce of blood. On account of the large amount of blood necessarily removed with the spleen, the child received an intravenous saline infusion of twelve ounces. The condition after the operation was good. Blood examination after operation: 3,860,000 red cells; 19,600 leucocytes; hemoglobin, 66 per cent. Differential count: polynuclears, 72; large lymphocytes, 20; small lymphocytes, 8. Within a few hours the temperature rose to 104° F., and the child died the following day.

The spleen immediately after operation weighed 490 gm. (body weight 10.5 kilos), and measured 18 by 9.5 by 5 cm. The general configuration was quite normal, though the organ was slightly elongated. The surface was smooth; the color, a peculiar brownish salmon. On section, the surface was mottled, due to the presence of transparent pearly areas mixed with minute hemorrhagic spots. The pearly areas measured from 0.5 to 1 mm. in diameter, and were raised slightly above the surface; they were from 2 to 4 mm. apart. There were no tubercles present, nor any large nodules or areas of necrosis, the entire surface presenting a mottled, homogeneous appearance.

AUTOPSY.

Autopsy was performed six hours after death by Dr. Thalhimer. Body well nourished; dusky subicteric color of skin; no pigmentation; no edema. Moderate rigor mortis and post-mortem discoloration. A sutured, surgical incision, 15 cm. long, is seen at the left upper quadrant of the abdomen.

Heart.—Normal size and color; tricuspid orifice admits index finger; tricuspid and pulmonary valves normal. Mitral valve just admits the little finger; slight edematous thickening at line of closure; aortic valves normal; coronaries normal; foramen ovale closed. Musculature is pale, greyish brown, with a translucent, hydremic appearance. A few fatty streaks are seen in the aorta, not continued in the thoracic or abdominal portions.

Lungs.—No adhesions; surfaces smooth; color is mottled blackish red and pink; same on section; crepitation throughout.

Larynx, Trachea, and Bronchi.—Normal.

Tracheobronchial Lymph Nodes.—Enlarged, from a few millimeters to 1.5 cm. in diameter. They are soft, and the color on the surface and on section is reddish grey.

Thymus.—Weight, 6.8 gm. It presents two soft, somewhat enlarged, lobes of pinkish grey color. The cut surface is lymphoid in character.

Thyroid.—Normal. Three parathyroid bodies, 3 mm. in diameter, are seen. These have a normal appearance.

Abdomen.—No ascites. The site of the spleen is marked by several ligatures; no oozing from stump. Slight amount of fibrin is seen overlying the stomach. Diaphragm at level of fifth rib on the right, and sixth rib on the left side.

Liver.—Weight, 730 gm.; size, 20 by 13 by 6.5 cm. It extends 6 cm. below the free border of the ribs. The surface is smooth and glistening, and the color is light pinkish yellow; the capsule is not thickened. On section the same color is seen; the lobules are but poorly outlined, and some diffuse grey areas are noted. A few white irregularly shaped areas are present, varying in size from 2 mm. to 1 by 1.5 cm. in diameter. They are situated around the medium sized blood-vessels and slightly raised above the surface. Their borders are not well defined, and the outlines of the lobules can barely be determined in them. The hepatic vessels and portal vein are normal. At the hilus are about twelve enlarged lymph nodes, from 1 to 2 cm. in diameter. These are very friable, semi-translucent, and of light brownish grey color; on section the same color is seen and they are quite succulent in character. The gall bladder and bile passages are normal.

Kidneys.—Weight, 60 gm. each; color, purplish red; capsules not adherent; surfaces are smooth, showing a few remains of fetal lobulation; normal on section.

Adrenals.—Normal in size and appearance.

Esophagus.—Normal.

Stomach.—Mucosa is pale, otherwise normal.

Intestines.—Mucosa of duodenum is pinkish grey and studded with pearly grey follicles, from 1 to 4 mm. in diameter, projecting about 0.5 mm. above the surface. These follicles become less and less in number and finally disappear 30 cm. below the pylorus. A hyperplastic Peyer's patch, 2 by 0.5 cm. in size and pinkish grey in color, is present 160 cm. below the pylorus. Towards the cecum

these patches become larger and more numerous, but many of smaller size are seen as well. Many Peyer's patches are bright red in color. The last 5 cm. of the ileum are so studded with these patches that the normal mucosa is barely visible. The large intestines are normal.

Abdominal Lymph Nodes.—All mesenteric and retroperitoneal lymph nodes are enormously enlarged, many of them being from 1 to 2 cm. in diameter. They are similar in appearance to those described at the hilus of the liver. A few lymph nodes are seen about the pancreas. These are about 0.5 cm. in diameter and have a bright red color.

Pancreas.—Normal.

Splenic Vein.—Enlarged to about three times its normal diameter; contains fluid blood; intima smooth and glistening.

Splenic Artery.—Similarly enlarged, but otherwise normal.

Inferior Vena Cava.—Normal.

Iliac Lymph Nodes.—Enlarged and similar to those described at the hilus of the liver.[9]

Bone Marrow of Tibia.—Color, bright red with small white dots.

Brain and Spinal Cord.—Not examined (no permission).

Two monkeys (*Macacus rhesus*) were inoculated with an emulsion of the iliac nodes in saline solution.[10] About 5 c.c. of the emulsion were injected into the splenic vein of one monkey. This animal died fourteen weeks later and an enormous dilatation of the stomach was found, but sections from the spleen and liver were normal. The second monkey received a similar amount of the emulsion in the femoral vein. This animal has remained well up to the present time (about four months) and shows no abdominal enlargement or tenderness.

MICROSCOPIC EXAMINATION.

Smears made from a freshly cut surface of the spleen shortly after its removal were fixed while still moist in both acetone and methyl alcohol and also by heat. Various stains and chemical reagents were used to ascertain the nature of the protoplasm of the large cells, but no specific reactions were obtained by any of the methods. The cells present the general appearances described by all previous observers, but the peculiar streaked, wrinkled, or vacuolated character is not as well marked in fresh smears as in the tissues after fixation and hardening. In the smears the outline of the cell also is quite regular, the shrunken appearance entirely absent, and the nucleus fairly large and round. The size of the large cells as compared with the erythrocytes and lymphocytes is well seen in

[9] Several of the iliac nodes were removed under aseptic precautions for experimental purposes.

[10] I wish to thank Dr. Leo Buerger for his kindness in making these inoculations for me.

figure 1. Many of the cells have a finely stippled protoplasm, but no granules or pigment particles can be demonstrated. A few cells of very large size are seen; one of these measures 71.7 by 100 microns in diameter, and contains thirteen nuclei.

In the smears the erythrocytes are spread and scattered over the entire field, and it frequently appears as though they were situated within the large cells. A careful examination, however, shows that the blood cells are simply adherent to the large cells, no true evidences of phagocytosis being present.

Frozen sections were made from the fresh spleen, but no distinctive reactions could be found in the large cells with the use of various reagents and stains.

Pieces of the spleen, as well as of all the organs obtained at autopsy, were fixed in Zenker's fluid, formalin, alcohol, Orth's fluid, and acetone, and sections were cut in celloidin and paraffin. A large variety of stains were employed, including the connective tissue and reticulum methods of Mallory, Verocay, and Bielschowsky.

Spleen.—The sections are almost identical with those previously studied in two adult cases, but some distinctive features, probably due to the comparatively shorter duration of the disease, require further description. When a section is examined with the low power, one notices many large, round or oval spaces with well defined outlines. These are the venous sinuses, each of which is more or less distended with blood, and rendered conspicuous by its comparatively empty lumen (figure 2). With the high power a peripheral layer of large cells is seen closely united to the wall and apparently arising from the endothelium of the sinus. Some of the cells are detached and are seen lying free in the lumen of the sinus surrounded by blood cells (figure 3). The sections also contain a large number of similar alveolar spaces filled with the same type of large cells, but without any endothelial lining. The large cells are identical with those found in all the previous cases, and require no further description. In most of the sections there are strands of normal pulp cells situated between the alveoli, as well as apparently normal capillaries (figure 4).

Here and there are a few cells of a different character, not present in our two earlier cases. The cell body has the same general

appearance and size as the large cells, but even with the low power the nucleus is so large and deeply stained that the cell attracts attention at once. Under higher magnification the nuclear substance is seen to be irregular in outline and composed of from three to five or more nuclei fused into one mass, and rich in chromatin. As no karyokinesis is present in the sections, it is thought that these cells may possibly represent some stage of amitotic division, though they bear a striking resemblance to the megakaryocytes in the marrow.

The Malpighian bodies are of enormous size in this case. In the previous cases studied, these structures were few in number and usually normal in size and appearance. In the present case the average diameter of these bodies is from 0.6 to 1.04 millimeters, the normal size being from 0.2 to 0.7 of a millimeter. One of the larger of these bodies, oval in form, measures 1.5 by 0.8 of a millemeter, and the keimcentrum, 0.74 by 0.28. As in the previous cases, isolated groups of large cells are seen between the lymphoid cells of the follicles, often near the peripheral part.

In some of the keimcentra there are small masses of hyaline material, also atypical lymphoid cells, but no distinct cell division is seen. In many of the keimcentra the small deeply staining tingible bodies of Flemming (18) are found. These are contained in large cells which resemble the other large cells in size and shape. Many have nuclei and also contain the non-stainable pigment granules described by Flemming. These cells have all the appearances of phagocytes but they are limited entirely to the keimcentra. There are no other evidences of phagocytosis and no pigmentation. The material fixed in acetone was examined for erythrocytes in the large cells, but with negative results. Throughout the sections are mononuclear cells with eosinophile granulations. These are somewhat larger than the lymphoid cells, and the nucleus is often small and compact. Others have a larger vesicular nucleus and resemble typical myelocytes.

Sections stained for connective tissue and reticulum are of great interest. With the Mallory, Van Gieson, and Verocay methods, the connective tissue is seen prominently outlining the alveoli, and smaller fibers may be seen in other situations as well. With the Bielschowsky method, however, the general distribution of the retic-

ulum and the ramifications of the smallest fibrils may readily be studied. With the low power it is seen that all alveoli are surrounded by fairly coarse fibers (figure 5). The network is exceedingly complex and difficult to trace, ramifying as it does in all directions; but after a critical examination it may positively be stated that no reticulum fibers are ever seen within the lumen of the venous sinuses. In the thinnest paraffin sections one occasionally finds a parallel row of six or eight short and coarse fibers radially placed at the margin of a sinus. These are branches of the enveloping reticulum, apparently projecting into the sinus, but with careful study these fibers may be traced running in another plane above or below the sinus and never into its lumen. The sinus wall is invariably intact.

In the other alveolar spaces whose outlines are usually as well defined as the venous sinuses, and which at first glance may be mistaken for the latter, fine fibrils are often seen. In these situations the reticulum consists of short, rather blunt fibers projecting into the alveolar space, or, of very delicate fibrils apparently attached to the large cells and to the alveolar wall. Between the alveoli the fibers are present in such enormous numbers that it would be impossible for any cells to be present and escape being surrounded by this network. Therefore it is impossible to deny that a close relation exists between the reticulum and the large cells, even though one cannot find evidences of transitional stages between them.

The reticulum encircles the Malpighian bodies in a very regular and uniform manner, the individual fibers being short and delicate, as a rule. Three or four layers of fibers, somewhat separated from each other, are usually seen. Fibers are also found between the lymphoid cells of the Malpighian body and in the keimcentrum, but always in small amount. The fibers do not appear to be connected with either the peculiar cells described in the keimcentrum, or the large cells situated between the lymphoid cells, though it must be admitted that such a relation is possible.

There are no areas of degeneration, necrosis, or hemorrhage in the spleen, and no evidences of a tuberculous process.

Lymph Nodes.—The degree of involvement varies considerably

in the different groups of nodes, but the type of lesion corresponds, as a rule, with that described in other cases. The iliac, mesenteric, and retroperitoneal nodes, together with the nodes at the hilus of the liver, show an advanced process, while the bronchial and pancreas nodes, as well as those found along the splenic vein, present a much earlier lesion.

The retroperitoneal and mesenteric nodes show the greatest involvement. The follicles are nearly or entirely obliterated, but considerable adenoid tissue still remains in the medullary strands. The connective tissue framework is apparently normal, but in many places there are fibers which appear to divide the sinuses into rather distinct alveoli of round or oval shape. As a result, portions of the node closely resemble the pictures seen in the spleen. The lymph sinuses are easily recognized and filled with large cells. The endothelial cells of the sinuses are readily seen on the whole, and the large cells appear to be closely related to them. The subcapsular sinuses and those along the coarser trabeculæ are moderately dilated and filled with lymphocytes, erythrocytes, and large cells. The individual large cells are clearly and definitely outlined, and there is no tendency in this node towards the formation of extensive cellular masses or the fusion of adjacent cells (figure 6). No pigment is present and evidences of phagocytosis are wanting. Eosinophile myelocytes are found in all the sections. With Bielschowsky's method the reticulum is clearly seen, the general arrangement being similar to that described in the spleen. The alveolar spaces, as a rule, are distinctly outlined, but the fibers do not seem to have any apparent connection with the cells within. In some places, however, the same relationship exists between the reticulum and the cells, as was noted in the spleen.

The lymph nodes at the hilus of the liver show the lesion in an advanced degree, mostly in the medullary portions, and an alveolar arrangement similar to that noted in the retroperitoneal nodes is present. The follicles, however, are quite different in these nodes. They are present in the cortical zone in apparently normal numbers, but each follicle is strikingly prominent on account of the hypertrophy of the keimcentrum. The tingible bodies of Flemming are seen here in enormous numbers, and on account of their prominence

a detailed description may be permitted. The cells in which these bodies are found are round, and measure, on an average, twenty-two microns in diameter, but a cell is occasionally found showing an ovoid or fusiform outline. The cell body consists of an apparently slightly granular cytoplasm in which a rather large, oval, vesicular nucleus containing one nucleolus is seen. The nucleus stains readily, but the cytoplasm stains but lightly or not at all. From three or four to twenty or more of these cells may be present in a keimcentrum. The tingible bodies show considerable variation in size and shape. The diameter varies from 1.45 to 3.8 microns. Some of the smaller bodies are quite spherical in outline, others show a ring shape with a faint circular outline on one side and a crescent shaped mass of nuclear substance on the opposite side. A few of the ring shaped bodies have a small mass on one side and a small dot directly opposite, while others are divided in a biscuit shape. These bodies stain intensely with all basic dyes, and from six or eight to fifteen or more may be found in a single cell (figure 7). Besides these tingible bodies, many of the cells contain minute round granules of a yellowish color (cytomicrosomes), which cannot be stained. None of these cells contain any elements showing cell division, though the keimcentrum is the seat of many lymphocytes with the customary mitotic changes. Many sections were carefully examined to discover, if possible, the presence of these cells in other situations, but as the results were always negative, it was finally decided that the cells are confined to the keimcentra. It may be stated in passing, that the resemblance of these cells to protozoa is most striking at times, but as they have occasionally been found in other conditions, this opinion is not seriously entertained. That these cells are phagocytic in character seems definite, but their classification is most difficult. Whether they represent a peculiar type of lymphocyte or a reticulum cell with peculiar properties cannot be stated definitely at this time.

The distribution of the reticulum is similar to that seen in the nodes previously described. No pigment can be discovered in these nodes. Myelocytes with eosinophile granulations are seen throughout the sections.

The iliac nodes are similar to the retroperitoneal nodes, except

that no typical alveolar arrangement is seen, and the individual large cells are less distinct on account of fusion into extensive cellular masses. In the cortical zone a fairly large number of follicles are seen. The tingible bodies are present in the keimcentra in small numbers, but they do not seem to be enclosed in the peculiar type of cell described above. The nodes are somewhat congested and minute hemorrhagic areas are seen. No pigment can be found and evidences of phagocytosis are absent. Myelocytes with eosinophile granules are present. The reticulum has the same relation to the large cells as noted in the retroperitoneal nodes, and its distribution in the follicles and keimcentra appears normal (figure 8).

The lymph nodes along the splenic vein present quite a different picture, the lesion being much less advanced than in the nodes just described. With the low power the appearance is that of a fairly normal node showing a moderate degree of acute congestion. The follicles are well preserved, though the keimcentra appear somewhat large and prominent. The capsule and trabeculæ are normal; the sinuses are slightly dilated. With higher amplification one sees a large cell here and there between the pulp cells. With a four millimeter objective possibly four or five cells may be seen in some fields; in others there are none. In the vicinity of some of the follicles, however, a small group of eight or ten cells is seen occasionally. As a rule, however, the cells occur singly in this node and are therefore readily studied. Some of the cells are found in the lymph sinuses, but the largest number are irregularly distributed between the pulp cells. The reticulum in these nodes seems to have an intimate relationship to the large cells, for a small fibril appears to be attached to most of the cells found in the pulp. In the sinuses, however, as a rule the cells are found lying free. Occasionally a cell seems to have some connection with the endothelium of the sinus. The reticulum in the follicles and keimcentra has no apparent connection with the large cells.

The keimcentra in these nodes resemble those described in the nodes at the hilus of the liver, but the large cells with the tingible bodies and granules are not as numerous. In addition to the Flemming bodies, many of the cells contain also remnants of erythrocytes and small particles of pigment, thus establishing the phago-

cytic nature of these cells beyond question. In some of the keimcentra large cells are found which appear to be identical with the typical large cells of the disease. In none of the other nodes examined was this resemblance so marked. Some of these cells contain pigment and erythrocytes, but no tingible bodies. Small masses of slightly granular protoplasm without definite shape, and staining faintly with eosin or picric acid, are seen in many of the keimcentra. Lymphocytes are also present, but no mitosis is seen in these cells.

The pigment in these nodes is quite abundant. It occurs in the form of rounded granules of a yellow or brownish color, and gives the reaction for hemosiderin. The pigment is never free, but is always situated in cells of various sizes. Some of these cells are so filled with pigment granules that the cell bodies are obscured. Others contain but a few granules and in these cells an oval vesicular nucleus is seen. Some of the cells also contain erythrocytes. Many of these cells bear a certain resemblance in size and outline to the typical large cells, but their identity is difficult or impossible to establish on account of the presence of the pigment. In close proximity to some of these cells, large cells are seen without any evidences whatever of phagocytosis. In addition to the eosinophile myelocytes which are also found in all the other nodes, these nodes contain quite a large number of myelocytes with neutrophile granulations and many mononuclear cells with coarse, deeply staining basophile granules. The latter are metachromatic in quality and resemble histogenic mast cells more than basophile myelocytes. The sections also contain polymorphonuclear cells with eosin granulations in moderate numbers.

The pancreas nodes show the presence of but few large cells. The trabeculæ are rather thick and prominent. Only a few follicles are seen, but in the keimcentra the Flemming bodies are present in the type of cell already described. In these nodes it is readily seen that the large cells are phagocytes, for many of them show the presence of pigment and erythrocytes most distinctly. The pigment shows a positive iron reaction. Eosinophile myelocytes are found in moderate numbers.

The bronchial nodes show a considerable number of large cells

diffusely scattered, also follicles with very prominent keimcentra in which the Flemming bodies are seen. The large cells are not phagocytic, and the only pigment seen is anthracotic in nature. Eosinophile myelocytes are also present in these nodes.

Bone Marrow.—The large cells are very numerous, and the sections show a degree of involvement which approaches that seen in adult cases of long duration. The cells occur mainly in extensive groups, and only here and there are isolated cells observed. The larger groups show considerable distortion of the cells due to compression. Vacuoles are quite common, and most of the cells are elongated and show the wrinkled and striated appearance so often described (figure 9). Phagocytic cells are not present, however, and no pigment is seen. The empty spaces previously occupied by fat cells are of moderate size. The reticulum shows a close relation to the large cells, and fibrils may be seen running in all directions between the cells (figure 10). The capillaries show no changes. Myelocytes of all types are found, as well as erythrocytes, normoblasts, and a few megakaryocytes. Eosinophile and neutrophile leucocytes are also present, but in small numbers. Mast cells are not seen. Small collections of lymphocytes representing the normal lymphoid tissue of the bone marrow are found in places. A careful search was made for the presence of true follicles and keimcentra as described by Hedinger,[11] and Oehme,[12] but the marrow is so much involved by the large cells that such structures could not be found in the small remnants of lymphoid tissue still remaining.

Smears were made from the fresh marrow at the time of the autopsy. These show the presence of large cells as well as all of the normal marrow cells. Phagocytes, however, cannot be found.

Liver.—Some portions of the organ are quite normal, while other parts show the characteristic lesion. With the low power the picture is that of a cirrhosis, the interlobular connective tissue being

[11] Hedinger, E., (*Frankfürt. Ztschr. f. Path.*, 1907, i, 527) found distinct follicles and keimcentra in the lower end of the femur in an idiot 1½ years old with status lymphaticus.

[12] Oehme, C., (*München. med. Wchnschr.*, 1909, lvi, 446) examined the marrow in the long bones of twenty-five children, mainly cases of rickets. In twelve cases follicles with keimcentra were found in otherwise normal red marrow. This condition occurred with and without a general lymphatic hyperplasia.

much increased in amount (figure 11). The large cells are found in abundance in the connective tissue spaces, but the individual cells are not easily recognized and are difficult to stain. The cells are compressed by the connective tissue fibers and have a flattened and wrinkled appearance. None of the cells are phagocytic, as far as can be determined, and pigment cannot be found in the liver. The lobules are quite normal, but some distortion is seen in the peripheral parts, due to the presence of wide bands of interlobular connective tissue. The individual liver cells are fairly normal, though a coarsely granular appearance is seen here and there in some cells. The sinusoids are more or less filled with large cells, and here the individual cells are quite well preserved, showing a more rounded and normal appearance (figure 12). Erythrocytes cannot be found in any of the cells. The intralobular veins and the branches of the hepatic artery show no changes. Several large cells are seen in the lumen of a large vein cut longitudinally. The bile ducts are not increased in number and show no changes. With Bielschowsky's stain the reticulum shows a rather dense mass of fibers between the lobules, and in the meshes individual large cells are seen (figure 13). In the lobules the reticulum forms a delicate network of fibrils between the columns of liver cells, but the large cells in the sinusoids are in no way connected with the fibrils.

The white areas in the liver noted at the autopsy show coagulation necrosis surrounded by a zone of fibroblasts. That part of the organ in which these areas are found is but slightly involved by large cells and is otherwise quite normal in appearance. There is no evidence of a tuberculous process in the liver. Sections of the portal vein show no lesion.

Intestines.—The follicles in Peyer's patches are markedly hyperplastic and the keimcentra are of enormous size. In each keimcentrum are hundreds of tingible bodies, many of which are seen to be situated in cells of considerable size. The latter do not appear to be the large cells of the disease, but a certain resemblance to them as well as to large lymphocytes is noted. The intestines show no other changes.

Thymus.—The adenoid tissue appears normal and the organ shows no histological changes. No large cells are present and no mitosis can be found.

The other organs examined were the thyroid, parathyroids, lungs, heart, kidneys, pancreas, and adrenals. No lesions of importance bearing on the disease were found.

DISCUSSION.

An analysis of the cases in the literature from the clinical standpoint would be of considerable value, but this aspect of the disease cannot be considered here. There are several features regarding the pathology and histogenesis that are of equal interest and importance. The interpretations made by the first observers who believed the process to be either an epithelioma or an endothelioma of the spleen are no longer accepted. Recent writers have attempted to discover, if possible, the origin and nature of the large cells. Whether the cells originate from endothelial cells or from the reticulum has incited much study and given rise to considerable discussion. The American writers declared that the cells arose from endothelium, and considered the process to be an endothelial hyperplasia, whereas the German authors decided that the cells were derived from the reticulum, although the possibility of an origin from the endothelium of the lymph sinuses in the nodes was mentioned by Risel. Marchand, who studied mainly the chemical composition of the cells, finally stated that the cells might be derived from either reticulum or endothelium. None of the authors could find positive evidences of direct transition of the cells from reticulum, and although the impression was firmly made that such must be the case, none of the investigators could deny the possibility of an endothelial origin. All authors agree, however, that the cells must originate in some structure found in the lymph adenoid tissue of the hemapoietic system.

After a study of the present case, in which the lesions in some situations were of an early type, it no longer appears certain that the large cells arise from the endothelium of the venous sinuses, as originally stated. Although they seem to be in close contact in many places with the endothelial cells, the latter are often differentiated, especially from the detached large cells in the sinuses. The endothelial cells usually have a more definite spindle shape and are smaller in size, and the cell body as well as the nucleus stains

much darker than the large cell. It also happens that directly beneath the large cells one frequently sees perfectly normal endothelial cells, and furthermore, transitional forms are never seen.

If it be assumed that the cells do arise from endothelium in the spleen, it is not difficult to account for their presence throughout that organ, if one accepts the opinion of an open vascular system in the spleen and the ease with which the elements in the venous sinuses can mingle with the pulp cells. Recent studies have demonstrated that gaps are found normally between the endothelial cells of the venous sinuses as well as in the circular fibers of the surrounding reticulum. When the organ becomes distended these gaps naturally become wider and allow cells to pass in either direction. According to Mollier (19), the reticulum of the pulp and the endothelial cells of the venous sinuses are intimately connected, and Rössle and Yoshida (20) state that reticulum cells cannot be differentiated in lymph nodes from the endothelium of the lymph sinuses. Therefore the presence of large cells within the vascular and lymphatic channels as well as their situation in the surrounding structures is easily comprehended. In the bone marrow, however, the conditions are quite different. It is difficult, for instance, to explain the situation of the large cells in the bone marrow, assuming that they originate from the endothelial cells in the vessels of the marrow, and there is no evidence that they are transported to the marrow through the blood stream.

Let us consider for a moment the questions that arise if we assume an origin of large cells from the reticulum. It is not difficult in that case to account for the presence of the cells in most situations where they are found, and the complex solutions offered by Risel as well as by de Jong and Van Heukelom to explain the presence of the cells in the venous sinuses of the spleen are no longer necessary, provided that the open vascular system is accepted. In the bone marrow the situation of the cells is readily explained if one assumes that they arise from the reticulum. In the liver the conditions are quite different, for lymph adenoid tissue is not present. That the cells are carried from the spleen to the capillaries of the liver through the portal system is accepted by all authors, but the presence of the cells in the meshes of the interlobular connective

tissue is a more difficult problem which will be referred to subsequently.

From observations made in this case, however, it does not appear conclusive to me that the large cells arise from the reticulum. As stated previously in the microscopic examination, there are situations in which the large cells and the reticulum are in close contact, but the transitional stages are never found. Risel acknowledges throughout his work that the absence of a transitional stage is an important fact. In my sections, furthermore, no evidences of reticulum could be found in any of the venous sinuses. The outlines were distinct and the contents readily studied, but the absence of the smallest fibrils was significant. Wherever the large cells formed dense masses, reticulum fibers were seen in intimate relation with the cells, but the impression was always obtained that this was simply a contact between the two and not a genetic relation.

If the large cells are not derived from either endothelium or reticulum, there remains but one other element in the lymphatic-hemapoietic system to be considered and that is the lymphocyte. Risel discussed the possibility of such an origin in his case, and thought at one time that the cells might be derived from lymphoid elements. He called attention to the large size of the lymphocytes in the follicles of the lymph nodes, but as they were smaller than the large cells and had a somewhat different appearance, and as he failed to discover transitional forms in the spleen or bone marrow, this opinion was finally abandoned and he decided that they arose, not from lymphocytes, but from the intrafollicular reticulum. Risel naturally mentions the fact that if the large cells were derived from lymphocytes, their presence in the general circulation might be expected. Their absence could be explained, he correctly states, on account of their large size, which would prevent them from filtering through the lymph nodes and the capillaries of the liver.

In the present case my attention was attracted to the marked hypertrophy of the follicles of the spleen and the large size of the keimcentra in this organ, as well as in some of the lymph nodes, and considerable time was devoted to the study of these structures. The keimcentra, especially in the nodes showing an early type of lesion, presented an appearance of unusual activity, and

contained many cells of a peculiar type which have already been described. It is true that these cells were limited to the follicles, but it seems reasonable to believe that some changes in size and appearance might occur after leaving the follicles. That these cells have phagocytic properties has already been mentioned, and the peculiar vacuolated appearance of most of the typical large cells often gives the impression of a generalized phagocytosis. Risel also describes the phagocytes in the spleen, nodes, and bone marrow in his case. We cannot assume, however, that all the large cells are actively phagocytic, for an intense anemia would quickly ensue. It is probable, nevertheless, that all the cells possessed phagocytic qualities at some stage of their existence. A mild degree of anemia is seen in many of the cases during the progress of the disease, and the bone marrow is always transformed into the red lymphoid variety. The presence of myelocytes in the spleen and nodes, as well as the multinuclear cells (megakaryocytes?) seen in the spleen, leads me to believe that an attempt at myeloid metaplasia is present in these organs, in the nature of a compensatory regeneration to balance the insufficiency of the marrow.

Whether the peculiar large cells in the keimcentra are specific lymphoid elements, whether they are derived from the reticulum cells of the keimcentrum, or whether they are cells of unknown origin, cannot be stated definitely. That the large cells of the disease are derived from them seems a reasonable assumption.

As previously stated, no follicles were found in the bone marrow, but such structures may have existed here originally, according to the researches of Hedinger and Oehme. The follicles are often destroyed or difficult to locate in other organs on account of a replacement by the large cells, and this may have occurred in the present case.

The lymph adenoid tissue in other parts of the body, such as the tonsils and follicles in the intestines, has never been involved by large cells in any of the reported cases. In the present case, however, a marked activity was noted in the follicles in Peyer's patches, and many of the peculiar cells seen in the keimcentra of other organs were found here. This seems significant in view of the fact that the lymphoid apparatus of the intestines bears a certain relation to

the hemapoietic system, and probably assists in the production of lymphocytes. The lesion was confined to the follicles, however, and the typical large cells were not present. It seems possible that the keimcentra in this situation are incited to an unusual state of activity, but that the process never reaches that stage where typical large cells are produced. The tonsils, unfortunately, were not examined in this case.

The resemblance of the large cells in the keimcentra to protozoa has already been mentioned. Risel also called attention in his case to the similarity of the large lymphocytes in the follicles to the protozoa described by Marchand and Ledingham (21) in kala-azar, and to the phagocytes found by Sauerbeck (22) in experimental trypanosome infection. The latter author declares that the phagocytes are derived from large lymphocytes in the spleen, lymph nodes, and bone marrow, as well as from the endothelial cells of the capillaries in the liver.

In this connection attention must be called to a recent work by Lintwarew (23) on the destruction of erythrocytes in the spleen and liver under normal and pathological conditions. The author states that certain phagocytes—erythrophages—exist in the spleen normally, and that blood cells are carried by them to the capillaries of the liver, where the blood pigment is changed to bile pigment in consequence of the destruction of the erythrophages. In pathological processes the phagocytosis is excessive, and the irritation caused by the products of disintegration in the periphery of the lobules produces a cirrhosis of the liver. The destruction of erythrocytes is compensated for by a change in the bone marrow to the red type. In a case of Banti's disease which Lintwarew examined, erythrophages were found in large numbers in the spleen and liver. The author places Banti's disease, primary splenomegaly, cirrhosis of the liver, and pernicious anemia in one group, and claims that these various diseases are forms of anemia differing from each other only in the intensity of the blood changes. The erythrophages originate in the follicles of the spleen and lymph nodes, (the latter were not examined by Lintwarew), and certain exogenous poisons, such as tuberculosis, syphilis, alcohol, and malaria, as well as endogenous toxins formed in the intestinal tract, cause an increased production of erythrophages.

On account of the rapid disintegration of these bodies and the difficulty in recognizing the erythrocytes in them, Lintwarew recommends that scrapings from the fresh organs be examined in normal saline solution, and that tissues be fixed in acetone. This was done in the present case. No phagocytes were found in the fresh scrapings, but here and there in some of the nodes a few were seen. The phagocytes were not found in the enormous numbers described by Lintwarew, and none could be discovered in the spleen or liver.

It is unfortunate that Lintwarew's observations on primary splenomegaly were limited to a study of Schlagenhaufer's paper and a comparison of the illustrations with his own sections. It is evident that a certain similarity exists between Lintwarew's photographs and the drawings in Schlagenhaufer's article, but it is also apparent that the vacuoles shown in the drawings were mistaken for erythrocytes by Lintwarew. However, it is quite possible that erythrophages are present in large numbers in primary splenomegaly at some stage of the disease, but it seems incredible, as stated above, that all the large cells are actively phagocytic. Inasmuch as the disease is a chronic one, it appears to me as though the phagocytosis could not be excessive for any considerable time. This view is also corroborated by the clinical picture as well as by the absence of a marked anemia. That the disease is related in some way, however, to the blood diseases cannot be denied, for the tendency to hemorrhages, the pigmentation of the skin and other organs, as well as the presence of myelocytes in the tissues, would tend to place it in that class. It remains for future observers to ascertain the presence of phagocytes in the various blood diseases, and to cultivate the fresh tissues *in vitro* for the same purpose.

SUMMARY.

As a result of this study it may be stated that primary splenomegaly of the Gaucher type is a distinct disease, related in all probability to the blood diseases. It begins usually at an early age, frequently affects several members of a family, and runs a chronic course.

The clinical manifestations are: pronounced hypertrophy of the

spleen, subsequent enlargement of the liver, absence of palpable lymph nodes, absence of jaundice and ascites, absence of characteristic blood changes, discoloration or pigmentation of the skin, and a tendency to epistaxis or other hemorrhages.

The lesions are found in the spleen, lymph nodes, bone marrow, and liver. These organs show the presence of iron-containing pigment, and large multinuclear cells with a characteristic cytoplasm. In the early cases peculiar large phagocytic cells arising from atypical large lymphocytes are found in the follicles (keimcentra) of the hemapoietic system. After leaving the follicles these cells possess phagocytic qualities for a certain period. As a result of the phagocytosis the cells enlarge, the nature of the cytoplasm changes, and the cells acquire a characteristic vacuolated and wrinkled appearance. The cells are carried from the spleen through the portal system to the liver, where they are destroyed. The irritation produced by this destructive process gives rise to an increase in the intralobular connective tissue.

The disease is eminently a chronic one, without any of the manifestations of malignancy, and always terminating as the result of some intercurrent affection.

The etiology is unknown, although a family predisposition to some toxic agent which causes an irritability of the follicles in the hemapoietic system probably exists. The possibility of some protozoan infection as an etiological factor must not be overlooked.

BIBLIOGRAPHY.

1. Gaucher, P. C. E., De l'epithélioma primitif de la rate, Paris, 1882.
2. Collier, W., *Tr. Path. Soc. London,* 1895, xlvi, 148.
3. Picou, R., *Bull. Soc. anat. de Paris,* 1895, lxx, 531.
4. Picou, R., and Ramond, F., *Arch. de méd. expér. et d'anat. path.,* 1896, viii, 168.
5. Bovaird, D., Jr., *Am. Jour. Med. Sc.,* 1900, cxx, 377.
6. Brill, N. E., Mandlebaum, F. S., and Libman, E., *Proc. N. Y. Path. Soc.,* 1904, iv, 143.
7. Brill, N. E., Mandlebaum, F. S., and Libman, E., *Am. Jour. Med. Sc.,* 1905, cxxix, 491.
8. Schlagenhaufer, F., *Verhandl. d. deutsch. path. Gesellsch.,* 1907, x, 77.
9. Schlagenhaufer, F., *Virchows Arch. f. path. Anat.,* 1907, clxxxvii, 125.
10. von Herczel, E., *Wien. klin. Wchnschr.,* 1907, xx, 127.
11. Marchand, F., *München. med. Wchnschr.,* 1907, liv, 1102.

12. Risel, W., *Verhandl. d. deutsch. path. Gesellsch.*, 1909, xiii, 252.
13. Brill, N. E., Mandlebaum, F. S., and Libman, E., *Am. Jour. Med. Sc.*, 1909, cxxxvii, 849.
14. Risel, W., *Beitr. z. path. Anat. u. z. allg. Path.*, 1909, xlvi, 241.
15. De Jong, R. de J., and Van Heukelom, J. S., *Beitr. z. path. Anat. u. z. allg. Path.*, 1910, xlviii, 598.
16. Brill, N. E., *Am. Jour. Med. Sc.*, 1901, cxxi, 377.
17. Reuben, M. S., *Am. Jour. Dis. Child.*, 1912, iii, 28.
18. Flemming, W., *Arch. f. mikr. Anat.*, 1885, xxiv, 50.
19. Mollier, S., *Arch. f. mikr. Anat.*, 1911, lxxvi, 608.
20. Rössle, R., and Yoshida, T., *Beitr. z. path. Anat. u. z. allg. Path.*, 1909, xlv, 110.
21. Marchand, F., and Ledingham, J. C. G., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1904, xlvii, 1.
22. Sauerbeck, E., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1906, lii, 31.
23. Lintwarew, J., *Virchows Arch. f. path. Anat.*, 1911, ccvi, 36; *Ann. de l'Inst. Pasteur*, 1912, xxvi, 51.

EXPLANATION OF PLATES.[13]

Plate 78.

Fig. 1. Smear from spleen, acetone fixation, Giemsa's stain. Several large cells, some of which contain multiple nuclei of large size. The remainder of the field is occupied by darkly stained lymphocytes and erythrocytes. × 400.

Fig. 2. Section of spleen with prominent venous sinuses. Each sinus is distended by erythrocytes, and with the low power appears quite empty. The remainder of the field is composed of large cells in an irregular alveolar structure. A few small areas of normal pulp tissue are seen at the periphery. × 80.

Plate 79.

Fig. 3. Section of spleen showing the central portion of figure 2 under higher amplification. Segments of three venous sinuses showing the delicate walls and the apparent relationship of the large cells to the endothelium, the free cells being surrounded by erythrocytes. Three small capillaries in transverse section are seen in the center of the photograph; towards the left is a blood-vessel of larger size; above the vessels is an alveolus filled with large cells. × 400.

Fig. 4. Section of spleen showing alveoli filled with large cells. The central alveolus is surrounded by a zone of normal pulp cells, and to the right and left small capillaries in transverse section are indistinctly seen. × 400.

Plate 80.

Fig. 5. Section of spleen, Bielschowsky's silver method. The reticulum is seen surrounding all the alveoli (the larger spaces being venous sinuses), but no fibers are seen anywhere within the lumen of a sinus. The bundle of fibers seen

[13] The diameter of each photomicrograph is reduced about 1/10 in the process of reproduction.

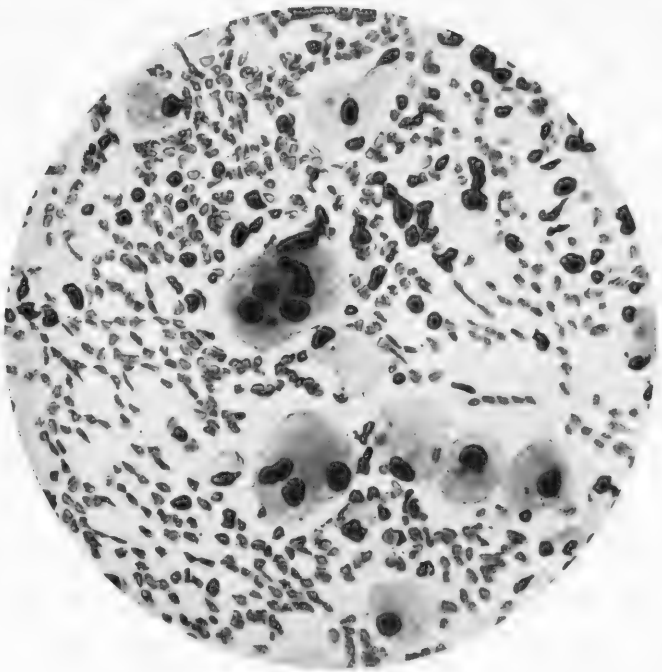


FIG. 1.

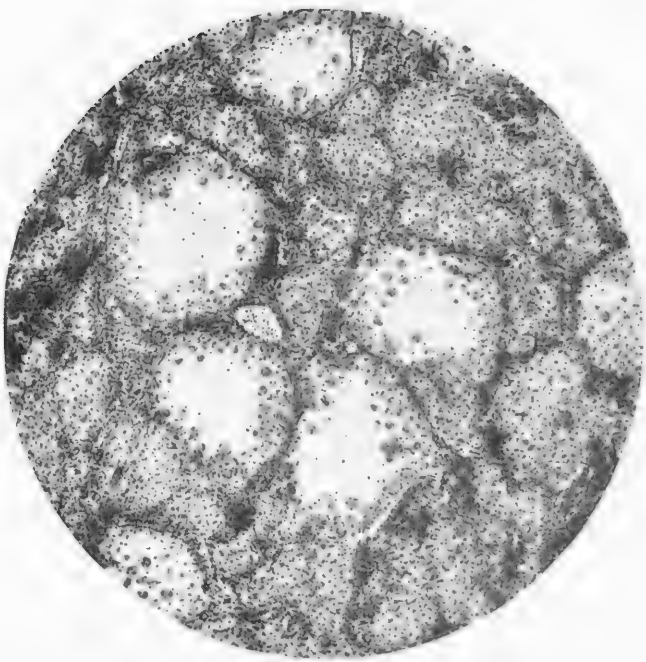


FIG. 2.

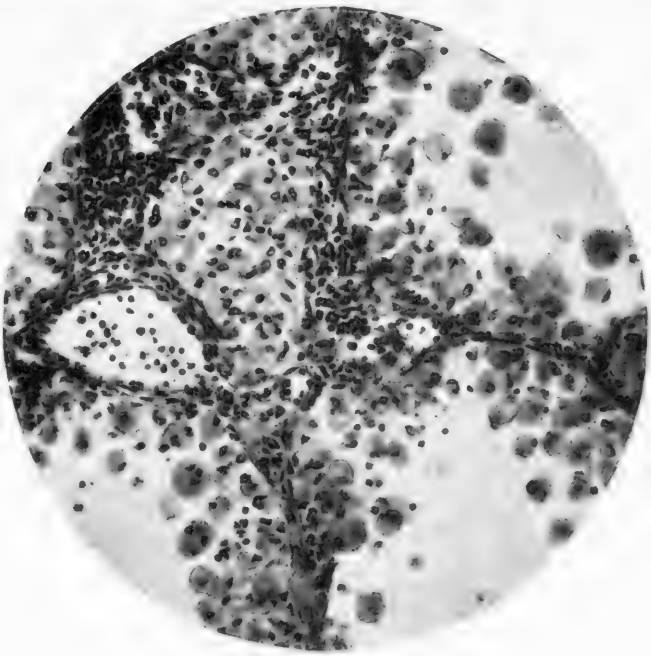


FIG. 3.

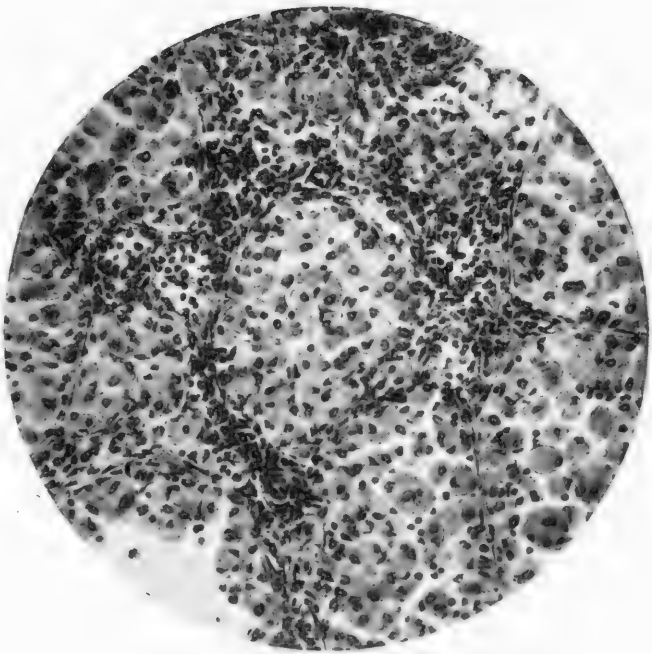


FIG. 4.

FIG. 5.

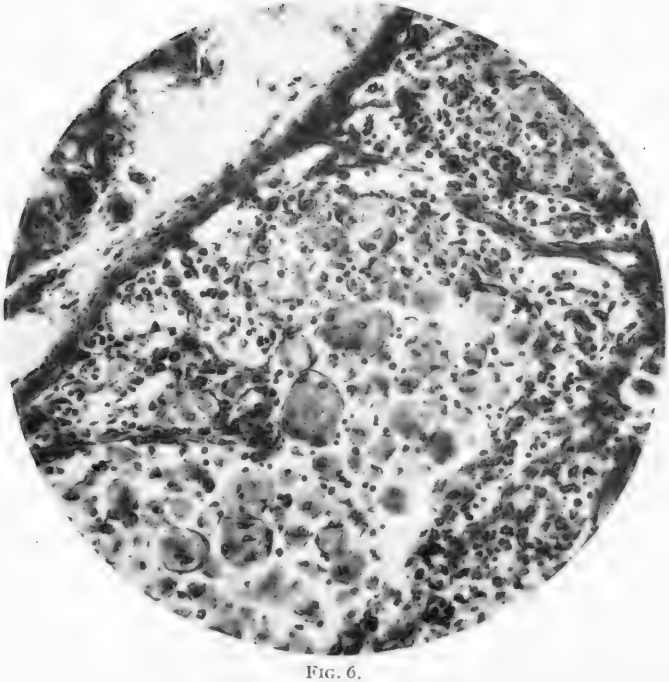


FIG. 6.

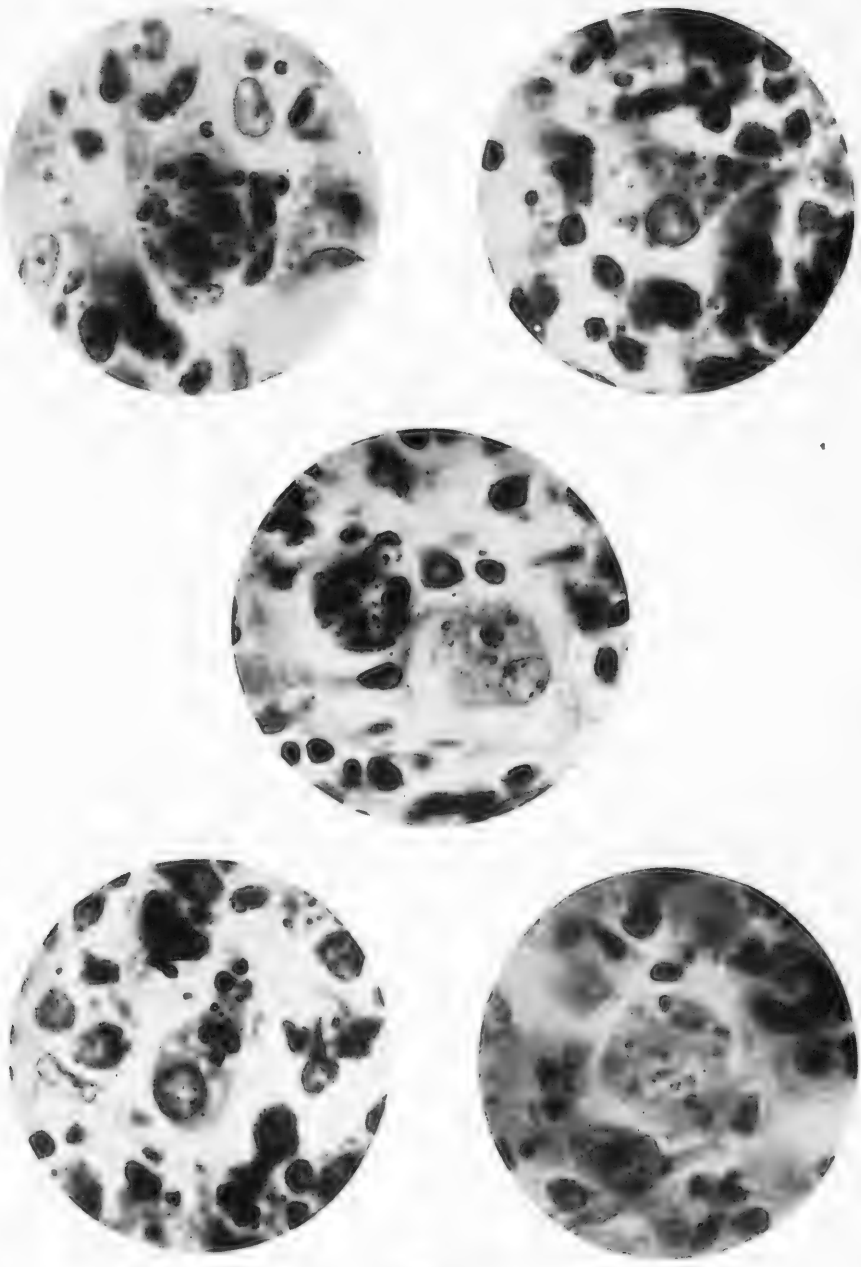


FIG. 7.

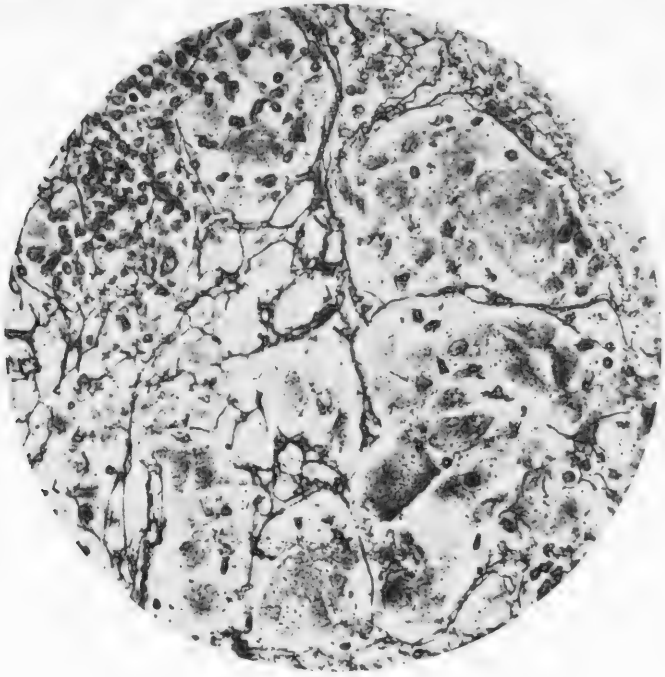


FIG. 8.

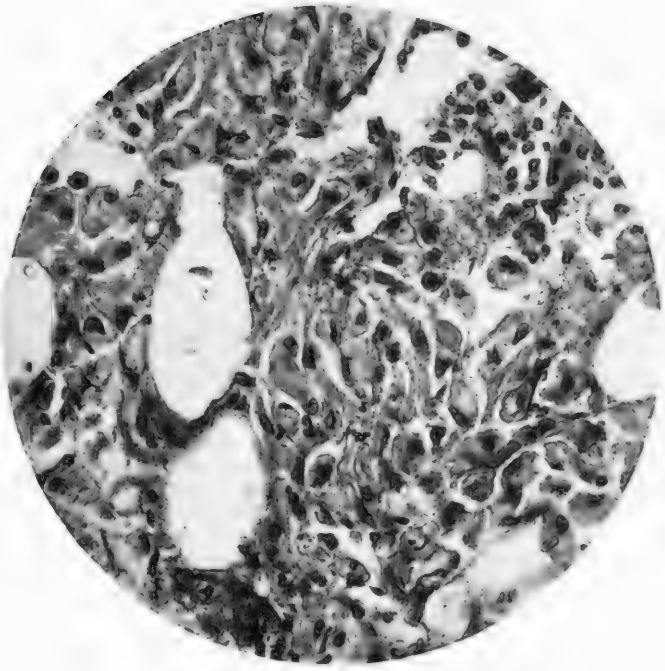


FIG. 9.

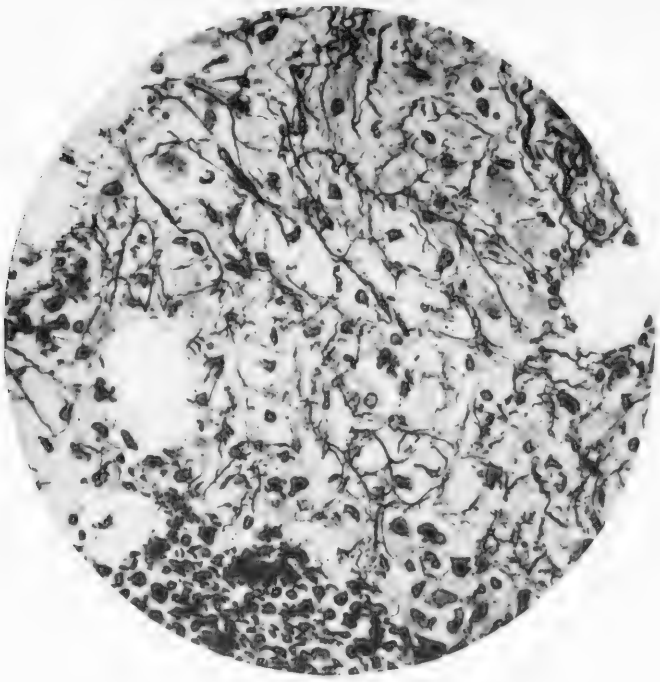


FIG. 10.

FIG. 11.

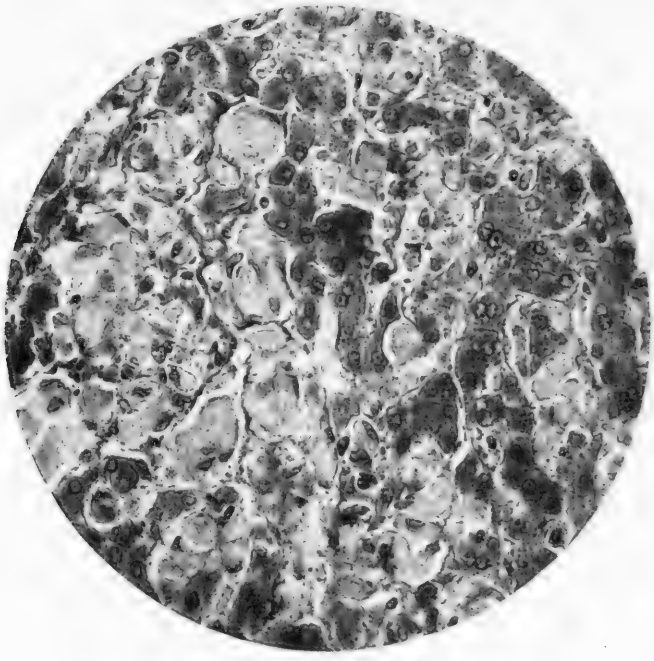


FIG. 12.

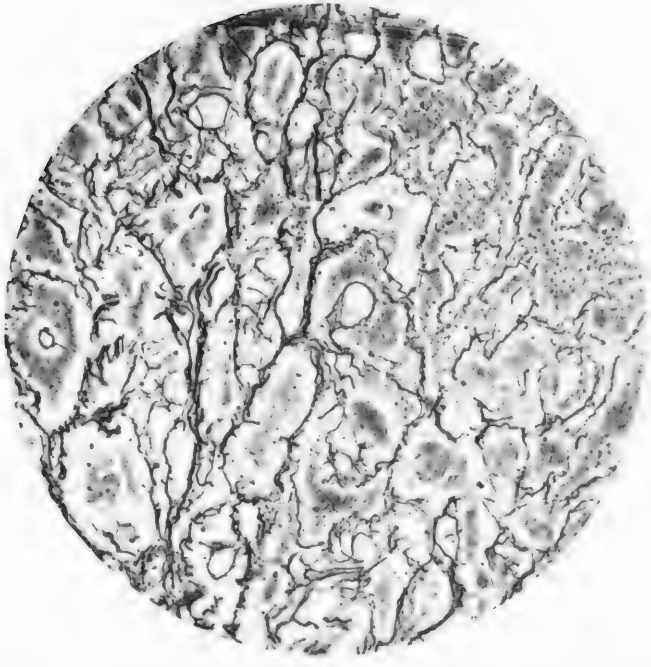


FIG. 13.

at the lower margin of the large sinus in the right upper part of the field does not enter the lumen but runs in another plane and encircles the sinus. × 80.

Fig. 6. Section of a retroperitoneal lymph node. Beneath the capsule seen at the left is a dilated sinus containing lymphocytes, erythrocytes, and a few large cells. In the central and lower portions of the field the cells are of large size and the contour of each cell is readily seen. Part of an alveolar space filled with large cells is seen above; fairly normal lymphoid tissue is present on the right. × 400.

Plate 81.

Fig. 7. Sections from the keimcentra of the lymph nodes at the hilus of the liver showing peculiar phagocytic cells containing Flemming's tingible bodies. In some of the cells the large nucleus is seen, in others the nucleus is either masked or out of focus. The finer details and contours of many of these bodies are difficult to show in a photograph. × 1,000.

Plate 82.

Fig. 8. Section of lymph node, Bielschowsky's method. In the upper part are two alveoli surrounded by reticulum. The lower half of the field shows reticulum fibers and large cells with a certain indefinite relationship. Some fibers are seen at the left among lymphoid cells at the margin of a follicle. × 400.

Fig. 9. Section of bone marrow showing compression and elongation of the large cells, also the vacuoles and wrinkled appearance. Above and to the right is a small area of normal marrow elements. × 400.

Plate 83.

Fig. 10. Section of bone marrow, Bielschowsky's method. The entire field is composed of large cells and reticulum fibers, excepting an area of marrow cells at the lower margin. The large cells stain indistinctly, but the nuclei are readily seen. Reticulum fibers are seen between most of the cells. × 400.

Fig. 11. Section of liver, Bielschowsky's method, to show the distribution of the intralobular connective tissue. All the lighter areas represent bands of connective tissue, giving the appearance of a cirrhosis of high degree. The lobules are well preserved, but the peripheral portions are somewhat involved by the connective tissue. × 50.

Plate 84.

Fig. 12. Section of liver. The left side of the photograph shows the appearance of the large cells in the spaces of the interlobular connective tissue. On the right an area of normal liver cells is seen, also a few large cells in the sinusoids. × 400.

Fig. 13. Section of liver, Bielschowsky's method, showing a field similar to figure 12. On the left the large cells are seen in the intralobular connective tissue spaces entirely surrounded by reticulum. On the right the delicate fibrils are seen between the columns of liver cells. × 400.

THE SELECTIVE ACTION OF GENTIAN VIOLET ON CLOSELY RELATED BACTERIAL STRAINS.*

By JOHN W. CHURCHMAN, M.D., and W. HOWARD MICHAEL.

(*From the Pathological Laboratory and the Biological Division of the Laboratory of the Medical Clinic, Johns Hopkins University, Baltimore.*)

Plate 85.

In a previous communication[1] a selective bactericidal property possessed by gentian violet was described, and observations were reported to indicate the nature of this chemical affinity as exhibited in a study of a large number of representative bacteria.

It was shown that bacteria could be sharply divided into two groups by their behavior toward gentian violet, either by staining the organisms directly, and then transplanting them to ordinary media, or by growing the unstained organisms on media containing the dye. In one of the groups of bacteria thus distinguished, gentian violet prevented growth; in the other it was without effect. The first group contained such organisms as *B. subtilis, B. anthracis, B. mycoides, M. aureus, M. albus,* Streptococcus, and *B. diphtheriæ;* in the other group fell *B. typhosus, B. paratyphosus, B. coli communis, B. pyocyaneus, B. prodigiosus, B. choleræ suis,* and many others. Organisms whose growth was inhibited by the dye were referred to as violet positive, while those whose growth was uninfluenced were termed violet negative. The selective action of the dye was well demonstrated by the use of divided plates, so made as to contain plain agar in one half and gentian violet agar in the other. A divided plate across which two violet positive and two violet negative organisms have been stroked is shown in figure 1, which illustrates the selective action of the dye and the technique of its demonstration. The effect of the dye on a violet positive organism is shown in figure 2, which represents a divided plate that has been stroked with seropurulent material from a suppurating parotitis in typhoid fever. On the plain agar side of the plate a number of discrete colonies of *M. aureus* are seen growing, while the gentian violet side of the plate has remained sterile, though the track of the inoculating needle is plainly marked on the plate by a film of dried bloody serum. In smears from the gentian violet half of the plate, no organisms were to be found.

It was not expected that closely related organisms would differ in their behavior toward gentian violet; and it was found, in general, by a study of the effect of the dye on 138 distinct bacterial species,

* Received for publication, August 16, 1912.

[1] Churchman, J. W., *Jour. Exper. Med.*, 1912, xvi, 221.

that nearly allied organisms were similarly affected by the stain. *Bacillus typhosus,* for instance, was uninfluenced in its growth by gentian violet; so also were *Bacillus coli communis, Bacillus paracoli,* and *Bacillus paratyphosus.* Again, *Bacillus prodigiosus* grew luxuriantly in the presence of the dye; so also did *Bacillus pyocyaneus, Bacillus violaceus,* and *Bacillus indicus.* On the other hand, the growth of *Bacillus subtilis* was absolutely prevented by gentian violet; and this was equally true for *Bacillus anthracis, Bacillus anthracoides, Bacillus megatherium, Bacillus pseudoanthracis, Bacillus mycoides, Bacillus mesentericus,* and *Bacillus liodermos.*

In a study, however, of five nearly identical strains belonging to the enteritidis group, the selective affinity of the stain was found to be so specific in its nature as to distinguish between different strains of very closely related organisms. On the growth of one member of this group, the dye exhibited a constant and complete inhibition; on the growth of the other four it was absolutely without effect. It is the purpose of this paper to report a careful study of the behavior of this group of organisms toward the dye.

The organisms included in this study came from the following sources:

(1) E. J. H. H., *B. enteritidis,* Johns Hopkins University stock; originally isolated by Dunham, of Cambridge, in 1900.

(2) E. 18, from the Museum of Natural History, New York; originally obtained from The Rockefeller Institute for Medical Research.

(3) E. 25, from the above Museum; originally isolated by Jordan, from a drainage canal in Chicago.

(4) E. 26, *idem.*

(5) E. 132, from the above Museum; originally from Kral's laboratory.

(6) E. 234, from the above Museum; originally isolated by Dunham, of Cambridge.

The characteristics of the bacteria are shown in table I. None of them was tested for agglutination with a known enteritidis serum. For the purposes of this paper, the important point is that the five organisms were identical, according to the usual tests, and that their characteristics were those described for the enteritidis group. What name is given to them is here immaterial. They were all motile, Gram negative, non-spore-bearing bacilli, which did not coagulate milk or liquefy gelatin. They fermented glucose,

mannite, maltose, and galactose, but did not ferment saccharose, lactose, raffinose, or inulin. E. 26 formed gas a trifle more slowly than the others, and from agar growths of E. 234 and E. J. H. H. homogeneous emulsions in salt solution were less easily made than from agar growths of the other four. But with these very slight exceptions, all six organisms were identical, according to the usual morphological, tinctorial, and cultural tests.

Toward gentian violet, however, a constant and striking difference in the behavior of these organisms was noted. E. J. H. H. was the member of the group first studied; and it was expected, from the previous studies of the colon and paratyphoid group, that gentian violet would have no effect on its growth. On planting it, however, on divided gentian violet plates, we were surprised to find that its growth was absolutely prevented by the dye. This observation was repeatedly made, and was then confirmed by studying the New York descendant of the same original Cambridge stock (E. 234), which was found to behave in exactly the same way. Not only did these two descendants of the same original stock refuse to grow across divided gentian violet plates, but their growth was also much hindered or entirely prevented by staining the organisms and then transplanting them to agar or broth. Moreover, they would not grow in broth containing the dye in a strength of 1 to 80,000. They behaved as all violet positive organisms do.

These findings as to E. J. H. H. and E. 234 led to a study of the four other enteritidis strains. The growth of these (E. 18, E. 25, E. 26, and E. 132) was found to be absolutely uninfluenced by the dye, either when applied directly to the organisms (by the method of vital staining) or when applied to the media on which they were grown. They were, in a word, typically violet negative, in striking contrast to E. J. H. H. and E. 234, which, as has been said, had been found to be violet positive. This specific, selective affinity is well illustrated in figure 3, which shows a divided plate that has been stroked with E. 234, E. 18, E. 25, E. 26, and E. 132. The growth of one (E. 234) will be seen to have been prevented by the dye; on the other four the stain has had no effect whatever. This finding was constant in the large number of transplants made. In other words, of five organisms, identical in all ordinary tinctorial, morph-

TABLE I.

Behavior toward gentian violet.			Ordinary morphological, cultural, and tinctorial characteristics.													
Growth on divided plates.	Growth in gentian violet broth.	Growth after staining with gentian violet.	Organism.	Morphology.	Motility.	Gram stain.	Litmus milk.	Gelatin.	Glucose.	Saccharose.	Lactose.	Mannite.	Maltose.	Galactose.	Raffinose.	Inulin.
Good growth on both sides	Good growth	Good growth	E. 18	Short bacillus; no spores	+	o	Initial acidity; no coagulation	No liquefaction	Fermentation	No fermentation	No fermentation	Fermentation	Fermentation	Fermentation	No fermentation	No fermentation
Good growth on both sides	Good growth	Good growth	E. 25	Short bacillus; no spores	+	o	Initial acidity; no coagulation	No liquefaction	Fermentation	No fermentation	No fermentation	Fermentation	Fermentation	Fermentation	No fermentation	No fermentation
Good growth on both sides	Good growth	Good growth	E. 26	Short bacillus; no spores	+	o	Initial acidity; no coagulation	No liquefaction	Fermentation	No fermentation	No fermentation	Fermentation	Fermentation	Fermentation	No fermentation	No fermentation
Good growth on both sides	Good growth	Good growth	E. 132	Short bacillus; no spores	+	o	Initial acidity; no coagulation	No liquefaction	Fermentation	No fermentation	No fermentation	Fermentation	Fermentation	Fermentation	No fermentation	No fermentation
No growth on gentian violet side	No growth	No growth, or very faint, slow growth	E. 234	Short bacillus; no spores	+	o	Initial acidity; no coagulation	No liquefaction	Fermentation	No fermentation	No fermentation	Fermentation	Fermentation	Fermentation	No fermentation	No fermentation
No growth on gentian violet side	No growth	No growth, or very faint, slow growth	E. J. H. H.	Short bacillus; no spores	+	o	Initial acidity; no coagulation	No liquefaction	Fermentation	No fermentation	No fermentation	Fermentation	Fermentation	Fermentation	No fermentation	No fermentation

ological, and cultural characteristics, one (E. J. H. H. or E. 234) was constantly gentian violet positive; while the other four (E. 18, E. 25, E. 26, and E. 132) were constantly gentian violet negative. These facts are recorded in table I.

TABLE II.

Agglutination Reactions with Typhoid Serum.

Organism.	Dilutions of serum.				
	1–100	1–200	1–400	1–800	1–1,600
B. typhosus	+++	+++	+++	+++	0
B. paratyphosus	0	0	0	0	0
E. 18	0	0	0	0	0
E. 25	0	0	0	0	0
E. 26	0	0	0	0	0
E. 132	+	0	0	0	0
E. 234	0	0	0	0	0
E. J. H. H.	0	0	0	0	0

In order to get additional light on the real specificity of this selective action, the organisms were further studied by the method of agglutination. Each one was first tested with a known typhoid serum, and agglutination in no case occurred (table II). Rabbits were then immunized against E. 18, E. 25, E. 26, and E. J. H. H.,[2]

TABLE III.

Agglutination Reactions with the Serum of an Animal Immune to E. 18.

Organism.	Dilutions of serum.				
	1–200	1–400	1–800	1–1,600	1–3,200
B. typhosus	0	0	0	0	0
B. paratyphosus	0	0	0	0	0
E. 18	+++	+++	+++	+++	+
E. 25	0	0	0	0	0
E. 26	0	0	0	0	0
E. 132	+++	+++	+++	+++	++
E. 234	+++	+++	+++	++	0
E. J. H. H.	+++	+++	+++	++	0

and the agglutinative properties of these sera were tested against *Bacillus typhosus, Bacillus paratyphosus,* E. 18, E. 25, E. 26, E. 132, E. 234, and E. J. H. H. The results of these experiments are shown in tables III, IV, V, VI, and VII.

[2] Attempts at immunization against E. 132 were unsuccessful.

TABLE IV.

Agglutination Reactions with the Serum of an Animal Immune to E. 25.

Organism.	Dilutions of serum.				
	1–200.	1–400.	1–800.	1–1,600.	1–3,200.
B. typhosus	0	0	0	0	0
B. paratyphosus	0	0	0	0	0
E. 18	0	0	0	0	0
E. 25	+++	+++	+++	++	+
E. 26	0	0	0	0	0
E. 132	?	0	0	0	0
E. 234	0	0	0	0	0
E. J. H. H.	0	0	0	0	0

TABLE V.

Agglutination Reactions with the Serum of an Animal Immune to E. 26.

Organism.	Dilutions of serum.				
	1–200	1–400	1–800	1–1,600	1–3,200
B. typhosus	0	0	0	0	0
B. paratyphosus	+	0	0	0	0
E. 18	0	0	0	0	0
E. 25	0	0	0	0	0
E. 26	+++	+++	+++	++	+
E. 132	0	0	0	0	0
E. 234	0	0	0	0	0
E. J. H. H.	0	0	0	0	0

TABLE VI.

Agglutination Reactions with the Serum of an Animal Immune to E. J. H. H.

Organism.	Dilutions of serum.				
	1–200	1–400	1–800	1–1,600	1–3,200
B. typhosus	0	0	0	0	0
B. paratyphosus	0	0	0	0	0
E. 18	+++	++	+	0	0
E. 25	+	0	0	0	0
E. 26	0	0	0	0	0
E. 132	+++	+++	+	0	0
E. 234	+++	+++	+++	++	+
E. J. H. H.	+++	+++	+++	++	+

It will be seen: (1) that none of the sera caused clumping with *Bacillus typhosus* or *Bacillus paratyphosus;* (2) that E. 25 and E. 26 showed no interagglutination, either with each other or with the other organisms studied; (3) that E. J. H. H. and E. 234 were identical in their serum reactions; (4) that E. 18, E. 132, and E. J.

H. H. showed some interagglutination. From these agglutination experiments we may conclude: (*a*) that the organisms studied did not belong to the typhosus or paratyphosus group; (*b*) that two of them (E. 18 and E. 25) were to be distinguished by agglutination from the other three; (*c*) that four of them (E. 18, E. 132, E. 234, and E. J. H. H.) were closely related, as indicated by their interagglutinations (table VII).

TABLE VII.

Summary of Agglutination Reactions.

Organism.	Sera.					
	Typhoid.	Paratyphoid.	E. 18.	E. 25.	E. 26.	E. J. H. H.
B. typhosus	++	0	0	0	0	0
B. paratyphosus	0	++	0	0	0	0
E. 18	0	0	+++	0	0	++
E. 25	0	0	0	+++	0	0
E. 26	0	0	0	0	+++	0
E. 132	0	0	+++	0	0	++
E. 234	0	0	++	0	0	+++
E. J. H. H.	0	0	++	0	0	+++

Briefly, then, this study was concerned with five stock organisms, identical in all morphological, tinctorial, and cultural characteristics. They all failed to agglutinate with known typhoid serum, and to produce serum in animals which would agglutinate either *Bacillus typhosus* or *Bacillus paratyphosus.* Yet one of these organisms (E. J. H. H.) was sharply and constantly violet positive; while on the growth of the other four the dye was absolutely without effect. The studies of the organisms by the method of agglutination showed the gentian violet affinity to be quite as delicate as the serum affinity. The two did not run absolutely parallel. But by the use of gentian violet it was possible to distinguish, with great precision and constancy, one organism from four others identical with it in all ordinary characteristics and possessing similar agglutinative properties.

So far as we are aware, no instance of chemical affinity of this degree of nicety has previously been observed. The observation, as such, is an isolated one; and in itself has only the small value attaching to observations of that kind. As regards, however, its

bearing on the general subject of affinity between chemical substances and microörganisms, the instance here reported is of significance for the following reasons: (1) It establishes the fact that chemical substances may be so specific in their selective affinity for microörganisms as to distinguish among strains of bacteria otherwise indistinguishable. The importance of this fact to bacteriology is obvious. None of the closely related organisms which offer difficulty to bacteriologists, as regards isolation in purity, are so nearly identical as the five strains of *Bacillus enteritidis* studied in this investigation; yet an aniline dye picked one of these strains out with great constancy and precision. That a substance will be found possessing a similar selective affinity between such bacteriologically troublesome organisms as *Bacillus typhosus* and *Bacillus coli* does not seem to be out of the question. The observation reported in this paper gives an important lead, and indicates that a systematic study of the anilines by the method of divided plates should be carried out, for light it may well throw on the easy differentiation of closely related bacteria. (2) It indicates also to what extent our ideas of bactericides must be modified by the conception of chemical affinity. The fact that a given bactericide kills a given organism does not justify the conclusion that it will kill all closely related organisms, or even all strains of the same organism. Indeed the behavior of bacteria toward gentian violet, and particularly the instance of it reported in this paper, gives experimental basis for the assumption of chemical fastness which has previously lacked clear confirmation. The members of the general bacterial group to which *Bacillus enteritidis* belongs (*Bacillus typhosus, Bacillus coli, Bacillus paracoli*), and the majority of strains of *Bacillus enteritidis* itself, are naturally fast toward gentian violet: but we have happened to come upon one strain which, though identical with the others in every other respect, lacks this natural fastness and will not grow in the presence of the dye.

EXPLANATION OF PLATE 85.

FIG. 1. The upper half of the plate contains gentian violet agar, the lower half plain agar. The plate has been stroked with *B. typhosus, B. anthracis, M. aureus,* and *B. coli,* from left to right in the order named. The dye has been without effect on the growth of *B. typhosus* and *B. coli,* while it has completely prevented the growth of *B. anthracis* and *M. aureus.*

FIG. 2. The upper half of the plate contains gentian violet agar, the lower half plain agar. The plate has been stroked with the seropurulent exudate from a parotitis occurring during typhoid fever. Many colonies of *M. aureus* are seen growing on the plain agar side; the gentian violet side has remained sterile. The track of the inoculating needle is plainly seen across the whole plate.

FIG. 3. The upper half of the plate contains gentian violet agar, the lower half plain agar. The plate has been stroked with E. 18, E. 25, E. 234, E. 26, and E. 132, from left to right in the order named. It will be seen that on the growth of four, the gentian violet has been without effect, while the dye has completely prevented the growth of E. 234.

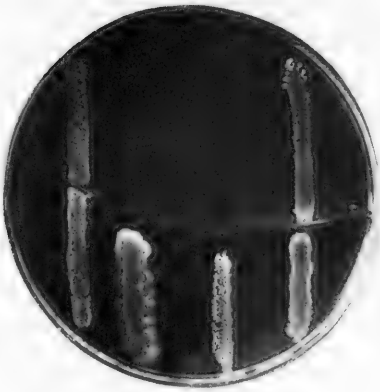


FIG. 1.

FIG. 2.

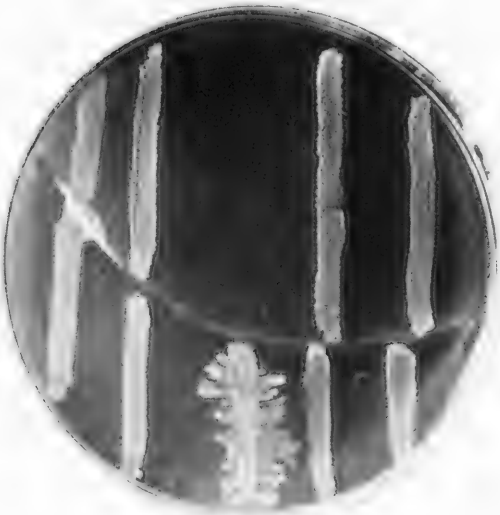


FIG. 3.

LYMPH FORMATION AND EDEMA OF THE LIVER WITH EXPERIMENTAL NEPHRITIS PRODUCED BY CANTHARIDIN.*

By EUGENE L. OPIE, M.D.

(*From the Department of Pathology of Washington University, St. Louis.*)

Experimental production of nephritis has offered a means of explaining the pathogenesis of nephritis and its associated phenomena not afforded by methods applicable to patients suffering with the disease. The possibility that changes in the liver have a part in the functional disturbances which accompany nephritis has been suggested by the occurrence of edema of the liver and gall bladder in association with nephritis produced by a substance capable of causing acute nephritis in man, namely, cantharidin. An attempt to analyze the factors upon which this hepatic edema depends has shown that the poison exerts an important influence upon the production and flow of lymph. Experiments with other renal poisons, as yet incomplete, have shown that they exert an important influence both upon the liver and upon the flow of lymph. The present article will be limited to a discussion of the changes caused by cantharidin.

The experimental studies of Heidenhain[1] upon lymph formation define two groups of substances which increase the production of lymph. Heidenhain's lymphagogues of the first order include a variety of protein substances such as peptone, extracts from the tissues of certain invertebrates, namely, extract of muscle of crab, extract of head and body of the leech, egg albumen, and other substances. When injected into the circulation of the dog, they cause an acceleration of the flow of lymph from the thoracic duct. This increase begins immediately after injection and lasts an hour or more; the lymph after injection contains an increased quantity of protein though its content in inorganic salts remains unchanged. Ten c.c. of an aqueous extract of crab meat may increase the flow of lymph sixfold and increase its protein content from 4.06 to 5.57 per cent.; a solution of peptone causes an even greater acceleration of the flow of lymph. Heidenhain's lymphagogues of the second order are crystalloid sub-

* Received for publication, August 30, 1912.

[1] Heidenhain, R., *Arch. f. d. ges. Physiol.*, 1891, xlix, 209.

stances; among those which he tested were grape sugar, sodium chloride, and sodium sulphate. These substances injected into the circulating blood may increase the flow of lymph from the thoracic duct and this lymph contains less protein than the normal lymph from the thoracic duct. Heidenhain cites experiments which indicate that the increased flow caused by lymphagogues of the first order is dependent upon the vital activity of the endothelial lining of the blood-vessels, whereas the action of crystalloids is dependent upon well known physical forces produced by changes in the concentration of the blood and tissue fluids. Lymphagogues of the first order cause no increase of urinary excretion, whereas crystalloids increase the flow of urine.

Starling[2] found that increase of lymph flow from the thoracic duct caused by peptone or by decoction of the muscle of crayfish may be almost completely prevented by ligation of the portal lymphatics. He attributes the entire increase to lymph derived from the liver and suggests that the small increase of flow which occurs after ligation of the portal lymphatics is referable to flow through those lymphatic channels which accompany the hepatic vein. Starling has found an increase of pressure in the portal vein following injection of so called lymphagogues of Heidenhain's first order, but since increased lymph flow continues after portal pressure has fallen, he believes that increased permeability of the capillaries of the liver has an important part in bringing about increased flow of lymph.

The capillaries of the liver, according to Starling, are more permeable than those in other tissues. Lymph obtained from the liver contains approximately from 6 to 8 per cent. of solids, whereas lymph from the intestine contains from 4 to 6 per cent., and lymph from the extremities only from 2 to 4 per cent. The capillaries of the liver are extremely sensitive to pressure, an elevation of pressure in the hepatic vein equal to 200 mm. of water produced by obstruction of the vena cava above the diaphragm causing an enormous flow of lymph derived from the liver.

The permeability of the hepatic capillaries demonstrated by the studies of Heidenhain, Starling, and others, gives significance to the occurrence of edema of the liver produced by cantharidin. This substance, it is well known, produces changes in the kidney accompanied by albuminuria. At the end of one hour after the injection of from 0.005 to 0.02 gm. of cantharidin into rabbits, Cornil and Brault[3] found an accumulation of material containing nucleated cells within the capsular space of the Malpighian bodies of the kidney. Later, according to their observations, cells lining the capsule are swollen and undergo desquamation. The cells of the convoluted tubules become swollen and their protoplasm is granular and vacuolated; within the lumina there is granular debris. With poisoning caused by repeated administration of small doses of cantharidin, round cells accumulate about the arteries. Eliaschoff[4] observed similar changes; in the capsular space were albuminous precipitate and cells which were regarded as swollen white corpuscles; swelling and desquamation of the capsular epithelium were found. The renal tubules exhibited degenerative changes.

[2] Starling, E. H., *Jour. Physiol.*, 1894, xvi, 224; 1894–5, xvii, 30.
[3] Cornil and Brault, Études sur la pathologie du rein, Paris, 1884, 149.
[4] Eliaschoff, I., *Virchows Arch. f. path. Anat.*, 1883, xciv, 323.

In white rats poisoned with cantharidin, Welch[5] found that cells accumulate in the capsular space. He does not regard these cells as swollen leucocytes which have emigrated from the blood-vessels nor as desquamated epithelial cells, but thinks that they are cells of the convoluted tubules which have been pushed back into the glomerular space. Lyon[6] has observed the same change; he has noted that the capsular space is devoid of contents in animals which survived longer than ten hours, the tuft filling it almost completely. Lyon found advanced degeneration in the tubules; the ascending limb of the loop of Henle may exhibit coagulative necrosis. The convoluted tubules are dilated and the inner parts of the cells undergo disintegration, leaving a basal part containing a nucleus which may stain deeply. The cells of the collecting tubules are swollen and granular, particularly in the larger tubules, and have frequently undergone desquamation into the lumen. Polynuclear leucocytes are often found between these cells. Casts are formed within the collecting tubules, particularly in the medullary rays. With less acute poisoning, lymphocytes may collect about blood-vessels.

Schlayer and Hedinger,[7] and more recently Pearce, Hill, and Eisenbrey,[8] using physiological methods have described experiments which they believe indicate the occurrence of two types of experimental nephritis, one caused by potassium chromate or uranium nitrate and referable to changes in the tubules of the kidney, and the other caused notably by cantharidin or arsenic and referable to changes in the glomeruli. Alteration of glomeruli is demonstrable by vascular reactions rather than by anatomical changes in the capillaries or epithelium of these structures. Animals with nephritis following the administration of potassium chromate or uranium nitrate eliminate an increased quantity of urine and exhibit a variety of reactions which demonstrate that the blood-vessels of the kidney retain their normal excitability to certain stimuli; the oncometer shows that transient sensory stimulation or administration of adrenalin causes the blood-vessels to contract with somewhat abnormal activity, whereas caffein or strong salt solution causes dilatation somewhat greater than that which occurs with administration of the drug in normal animals; phloridzin causes the polyuria and glycosuria observed after its administration to normal animals. In animals which have received cantharidin or arsenic, similar tests applied within from four to eight hours show that the vascular apparatus of the kidney has undergone profound alteration. A diminished quantity of urine is excreted; contraction and dilatation of the vessels under the conditions which have been named are diminished or absent; phloridzin causes no polyuria and no glycosuria.

After the injection of from 0.013 to 0.026 of a gram of cantharidin dissolved in ethyl acetate (one to two cubic centimeters of a solution containing 0.2 of a gram of cantharidin in fifteen cubic

[5] Welch, W. H., *Tr. Assn. Am. Phys.*, 1886, i, 171.

[6] Lyon, G., *Jour. Path. and Bacteriol.*, 1904, ix, 400.

[7] Schlayer and Hedinger, *Deutsch. Arch. f. klin. Med.*, 1907, xc, 1.

[8] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., *Jour. Exper. Med.*, 1910, xii, 196.

centimeters of ethyl acetate) into dogs ranging in weight from 2.5 to 10 kilos, I have found that the changes observed after death are fairly constant. Small animals are killed by 0.013 of a gram of cantharidin but larger animals survive this dose. Death with the hepatic changes which will be described may occur within five hours. The kidneys are slightly enlarged, succulent, and deep red throughout; the medullary rays are conspicuous, at times, as gray markings. Microscopical examination shows the profound alterations in the convoluted tubules described by Welch, Lyon, and others. Particularly noteworthy are the consequent changes within the glomeruli. The capsular space is dilated and contains both granular material, consisting of albuminous precipitate and hyaline droplets, and large nucleated cells, often in excellent preservation. The protoplasm of such cells is identical in appearance with that of the cells which compose the adjacent convoluted tubules. Their nuclei are often irregular in shape; at times the nuclei stain intensely, at times they remain almost unstained. Favorable sections passing through the junction of a glomerular capsule and its convoluted tubule show that the cells within the capsule are continuous with those of the tubule, which occasionally project as a rounded mass without obvious lumen through the neck of the capsular space. The epithelium of the capsule and of the glomerulus exhibits no alteration.

Coincident with the changes which occur in the kidneys, there are noteworthy changes in the liver and gall bladder. The liver is turgid and homogeneously deep red without lobular markings. Blood in great abundance escapes from its cut surface. The gall bladder is swollen and bluish gray in consequence of edema of its wall which may be two or three millimeters in thickness. The cellular tissue between the gall bladder and the liver is conspicuously edematous. Widely dilated lymphatics, often 1.5 millimeters in diameter, accompany the structures contained in the portal spaces and pass downward from the root of the liver through the gastrohepatic omentum to the regional lymphatic nodes which are situated at the base of the duodenal mesentery and in the gastrohepatic omentum along the upper margin of the splenic arm of the pancreas. Similarly dilated lymphatics connect adjacent nodes. The afferent lymphatics of the nodes usually contain blood tinged lymph and

end upon the surface of the node of which the peripheral sinus and medulla are stained red.

The lymphatic nodes which become swollen and edematous are as follows: (1) A large node situated at the base of the duodenal mesentery in contact with the superior mesenteric vein immediately below its junction with the splenic. (2) One, two, or occasionally more smaller nodes situated in the duodenal mesentery between its base and the duodenal arm of the pancreas; these nodes are occasionally in almost immediate contact with a group of large nodes at the base of the cecum. (3) From two to six lymphatic nodes situated in contact with the splenic vein in the mesenteric fold which is attached to the superior margin of the splenic arm of the pancreas. The largest of these nodes is that which is nearest the liver; it is in almost immediate contact with another large node, i. e., (1), which is adjacent to the superior mesenteric vein.

Microscopic examination of the liver demonstrates that edema of the gall bladder is part of a general edema of the liver. The tissue of the portal spaces is distended and its interstices contain albuminous coagulum; lymphatic vessels are dilated. There is similar edematous distension of the tissues above the central and sublobular veins. Dilated lymphatics contain coagulated lymph in which red blood corpuscles are numerous. Within the parenchyma of the lobules the endothelium of the capillaries is separated by wide spaces from the adjacent columns of liver cells, and in these spaces red blood corpuscles are numerous.

Edema of the gall bladder affects the serous more than other coats of the wall. In the serous coat and in the loose tissue in contact with the under surface of the liver are immensely dilated lymphatics which are constricted at intervals by circular valve-like folds.

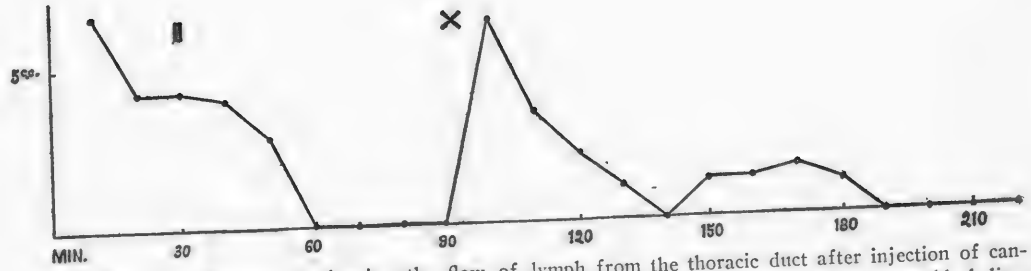
Enlargement of the regional lymphatic nodes of the liver is, in considerable part, the result of immense dilatation of the sinuses and especially of those within the medulla. Here red blood corpuscles, lymphocytes and large mononuclear cells are abundant. Cells showing various stages of disintegration are numerous; some have lost their nuclei whereas others contain deeply stained nuclear fragments. Within such sinuses, fibrinous coagulum forms a network

with fine meshes. It is obvious that fibrin formation has begun about injured cells, many of which still retain the characters of large mononuclear cells, for from these cells radiate in every direction fibrils of fibrin. In some instances, a pale, ill defined nucleus is surrounded by a radiating mass of fibrils which appear to have replaced the protoplasm of the cell. Within the medullary cords and in the cortex of the nodes, destruction of cells is further shown by abundant nuclear fragmentation occurring with greatest frequency in large cells with abundant protoplasm. In the germinating centers of the cortex, necrosis of cells with fragmentation of nuclei is particularly advanced.

Observations of Starling, which have shown that the increased flow of thoracic lymph caused by the action of Heidenhain's lymphagogues of the first order, namely, peptone, decoction of muscle from certain invertebrates such as crab, crayfish, etc., is derived from the liver, have suggested that edema of the liver caused by cantharidin is referable to increased flow of lymph from the liver. It is noteworthy, on the contrary, that administration of the substances employed by Heidenhain is not followed by edema. To determine the effect of cantharidin upon the flow of lymph from the thoracic duct, a cannula has been inserted into the duct exposed near its termination at the junction of the subclavian and jugular veins, and the flow of lymph has been measured during periods of ten minutes. Animals have been etherized during the entire course of the experiment which has been limited by the death of the animal to a period not exceeding three hours. At the end of this time, edema of the gall bladder has been repeatedly well advanced.

Diminution of the flow of thoracic lymph usually occurs within a period of from twenty to thirty minutes after intramuscular injection of cantharidin accompanied by gentle kneading of the injected muscle. In the two following experiments, the flow of lymph from the thoracic duct has been retarded and edema of the gall bladder has been noted at the end of two and of three hours respectively.

Experiment 1.—Dog, weighing 10,800 gm. A cannula was inserted into the thoracic duct; after observation of the lymph flow during 30 minutes cantharidin, 0.02 gm., was injected into the muscles of the thigh (text-figure 1).



TEXT-FIG. 1. Experiment 1, showing the flow of lymph from the thoracic duct after injection of cantharidin. Injection of cantharidin in this and in subsequent figures is indicated by a short heavy black line. At X the abdomen was opened and the intestines were disturbed.

Time.	Lymph in c.c. per 10 min.	Remarks.
12:40–12:50 P. M.	6.65	
12:50– 1:00 P. M.	4.30	
1:00– 1:10 P. M.	4.35	At 1:10 cantharidin, 0.02 gm., intramuscularly.
1:10– 1:20 P. M.	4.00	
1:20– 1:30 P. M.	2.70	
1:30– 1:40 P. M.	0.05	
1:40– 1:50 P. M.	0.02	
1:50– 2:00 P. M.	0.07	
2:00– 2:10 P. M.	0.01	
2:10– 2:20 P. M.	6.34	Abdomen opened and intestines disturbed.
2:20– 2:30 P. M.	3.40	Lymph is tinged with blood.
2:30– 2:40 P. M.	2.05	
2:40– 2:50 P. M.	1.10	
2:50– 3:00 P. M.	0.20	
3:00– 3:10 P. M.	1.20	
3:10– 3:20 P. M.	1.25	
3:20– 3:30 P. M.	1.50	
3:30– 3:40 P. M.	1.00	
3:40– 3:50 P. M.	0.30	
3:50– 4:00 P. M.	0.10	
4:00– 4:10 P. M.	0.10	
4:10– 4:20 P. M.	0.05	

The animal was killed at 4:27 P. M., 3 hours and 17 minutes after injection of cantharidin.

Autopsy.—The liver is red and the gall bladder edematous; conspicuous dilated lymphatics extend from the liver to the lymphatic nodes situated in the duodenal mesentery near its base and along the upper margin of the splenic arm of the pancreas. Microscopical examination of the liver shows edema, escape of red blood corpuscles, and dilatation of lymphatics. These nodes are enlarged and, with the surrounding tissue, edematous. The kidney is cloudy in appearance; the medullary rays are conspicuous.

Experiment 2.—A cannula was placed in the thoracic duct of a dog weighing 7,600 gm. A record of the quantity of lymph obtained during periods of 10 minutes is given below. After noting the flow during 30 minutes, 0.02 gm. of cantharidin was injected into the muscles of the thigh.

Time.	Lymph in c.c. per 10 min.	Remarks
11:05–11:15 A. M.	0.8	Lymph opaque and white.
11:15–11:25 A. M.	1.3	
11:25–11:35 A. M.	2.5	At 11:25 cantharidin, 0.02 gm., was injected.
11:35–11:45 A. M.	1.3	
11:45–11:55 A. M.	2.1	Lymph tinged with blood.
11:55–12:05 P. M.	1.3	

12:05–12:15 P. M.	1.0	
12:25–12:35 P. M.	0.6	
12:25–12:35 P. M.	0.6	
12:35–12:45 P. M.	0.35	
12:45–12:55 P. M.	0.9	
12:55– 1:05 P. M.	1.5	Lymph yellow and almost clear.
1:05– 1:15 P. M.	1.75	
1:15– 1:25 P. M.	2.1	
1:25– 1:35 P. M.	1.45	Death occcurred at 1:35 P. M.

Autopsy.—The liver is homogeneously deep red. The wall of the gall bladder is thickened by edema and there is edema of the gastrohepatic omentum, of the duodenal mesentery, and of the tissues about the ileocecal and mesenteric lymphatic nodes. Microscopical examination of the liver shows edema with escape of red blood corpuscles and dilatation of lymphatics. The hepatic regional lymphatic nodes in the duodenal mesentery near its base and along the upper margin of the splenic arm of the pancreas are enlarged and edematous. The kidney is deep red; the medullary striæ are conspicuous. Urine in the bladder contains no albumin and remains clear when boiled and acidified; it causes an atypical reduction of Fehling's solution but does not ferment.

In these experiments, administration of cantharidin has been followed by almost complete cessation of the flow of lymph. Although it has been possible to follow the changes which are caused by the poison during only three hours, advanced edema of the liver and gall bladder has been present. Such edema has been almost constantly observed when more prolonged action of the drug has caused death within from five to fifteen hours. The histological character of the edematous lesions has been described.

In experiment 1 at 2:10 P. M. the abdomen was opened and the intestines were manipulated in order to examine the bile ducts and the duodenal mesentery. This disturbance of the intestines was immediately followed by temporary discharge of a considerable quantity of thoracic lymph. Starling[9] has observed that pressure upon the intestines liberates abundant lymph. This temporary flow in experiment 1 serves to demonstrate that the otherwise almost total cessation of lymphatic flow is not the result of coagulation or other obstruction within the cannula or adjacent injured thoracic duct.

In the following experiment diminution of flow from the thoracic duct has followed almost immediately the administration of cantharidin but diminution of flow has been irregular and temporary; some edema of the gall bladder has been produced.

[9] Starling, E. H., *loc. cit.*, p. 224.

Experiment 3.—Dog, weighing 7,800 gm. A cannula was inserted into the thoracic duct. The foregoing experiment was repeated.

Time.	Lymph in c.c. per 10 min.	Remarks.
11:00–11:10 A. M.	4.9	Lymph grayish white.
11:10–11:20 A. M.	6.5	
11:20–11:30 A. M.	7.0	At 11:30 cantharidin, 0.02 gm., intramuscularly.
11:30–11:40 A. M.	3.4	
11:40–11:50 A. M.	1.7	Lymph faintly red.
11:50–12:00 A. M.	5.9	Lymph reddish in color.
12:00–12:10 P. M.	2.8	
12:10–12:20 P. M.	3.8	Lymph well marked yellow.
12:20–12:30 P. M.	4.5	
12:30–12:40 P. M.	2.1	
12:40–12:50 P. M.	4.4	Lymph deep yellow.
12:50– 1:00 P. M.	4.3	Lymph deep brownish yellow.
1:00– 1:10 P. M.	4.9	
1:10– 1:20 P. M.	4.3	
1:20– 1:30 P. M.	5.4	
1:30– 1:40 P. M.	6.3	
1:40– 1:49 P. M.	4.9	Died.

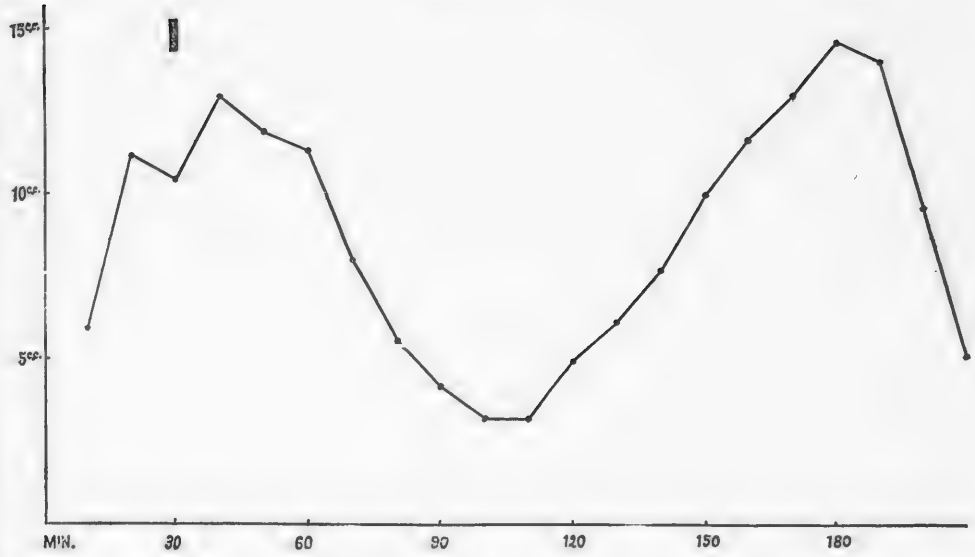
Autopsy.—The liver shows no noteworthy abnormality; the gall bladder is edematous in patches but is not uniformly swollen. The regional lymphatic nodes are swollen, red, and in part edematous. Dilated lymphatics pass from the liver to these nodes.

The urine from the urinary bladder contains a moderate amount of albumin. Reduction indicates the presence of 1.17 per cent. of sugar; the urine ferments.

In two experiments which will be described, there has been temporary diminution of lymph flow following injection of the drug, whereas in a third experiment (experiment 6) no diminution has occurred; yet in all of these experiments after about one and a half hours, there has been a greatly increased flow of thoracic lymph. With this active flow of lymph, no edema of the gall bladder or liver has occurred.

Experiment 4.—Dog, weighing 13,400 gm. A cannula was inserted into the thoracic duct with considerable difficulty (text-figure 2).

Time.	Lymph in c.c. per 10 min.	Remarks.
1:50–2:00 P. M.	5.8	Lymph grayish white.
2:00–2:10 P. M.	11.2	
2:10–2:20 P. M.	10.4	At 2:20 to 2:21 cantharidin, 0.052 gm., intramuscularly.
2:20–2:30 P. M.	12.85	



TEXT-FIG. 2. Experiment 4, showing the flow of lymph from the thoracic duct after injection of cantharidin.

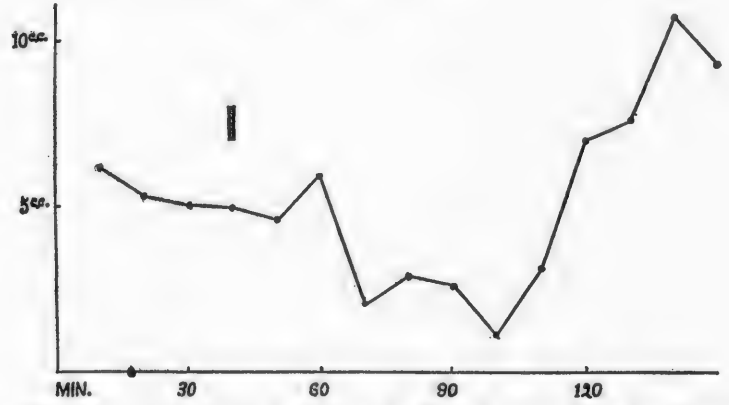
2:30–2:40 P. M.	11.7	
2:40–2:50 P. M.	11.3	Lymph blood tinged.
2:50–3:00 P. M.	7.9	
3:00–3:10 P. M.	5.5	
3:10–3:20 P. M.	4.2	
3:20–3:30 P. M.	3.2	
3:30–3:40 P. M.	3.2	
3:40–3:50 P. M.	4.8	
3:50–4:00 P. M.	6.1	
4:00–4:10 P. M.	7.6	Lymph deep red.
4:10–4:20 P. M.	9.9	
4:20–4:30 P. M.	11.65	
4:30–4:40 P. M.	12.9	
4:40–4:50 P. M.	14.6	
4:50–5:00 P. M.	14.0	
5:00–5:10 P. M.	9.6	
5:10–5:20 P. M.	5.2	Died.

Autopsy.—Liver is deep red. There is no edema of the gall bladder.

Experiment 5.—Dog, weighing 12,600 gm. A cannula was inserted into the thoracic duct (text-figure 3).

Time.	Lymph in c.c. per 10 min.	Remarks.
12:50–1:00 P. M.	6.2	Lymph opaque, white.
1:00–1:10 P. M.	5.3	
1:10–1:20 P. M.	5.0	
1:20–1:30 P. M.	4.9	At 1:30 cantharidin, 0.052 gm., intramuscularly.
1:30–1:40 P. M.	4.6	
1:40–1:50 P. M.	5.8	
1:50–2:00 P. M.	2.0	
2:00–2:10 P. M.	2.8	
2:10–2:20 P. M.	2.6	
2:20–2:30 P. M.	1.05	
2:30–2:40 P. M.	3.1	Lymph faintly blood tinged.
2:40–2:50 P. M.	7.1	
2:50–3:00 P. M.	7.6	Lymph blood tinged.
3:00–3:10 P. M.	10.7	Lymph yellowish.
3:10–3:20 P. M.	9.3	Lymph deeper yellow.
3:20–3:30 P. M.	4.2	Lymph bright yellow. Death.

Autopsy.—There is no edema of the gall bladder.



TEXT-FIG. 3. Experiment 5, showing the flow of lymph from the thoracic duct after injection of cantharidin.

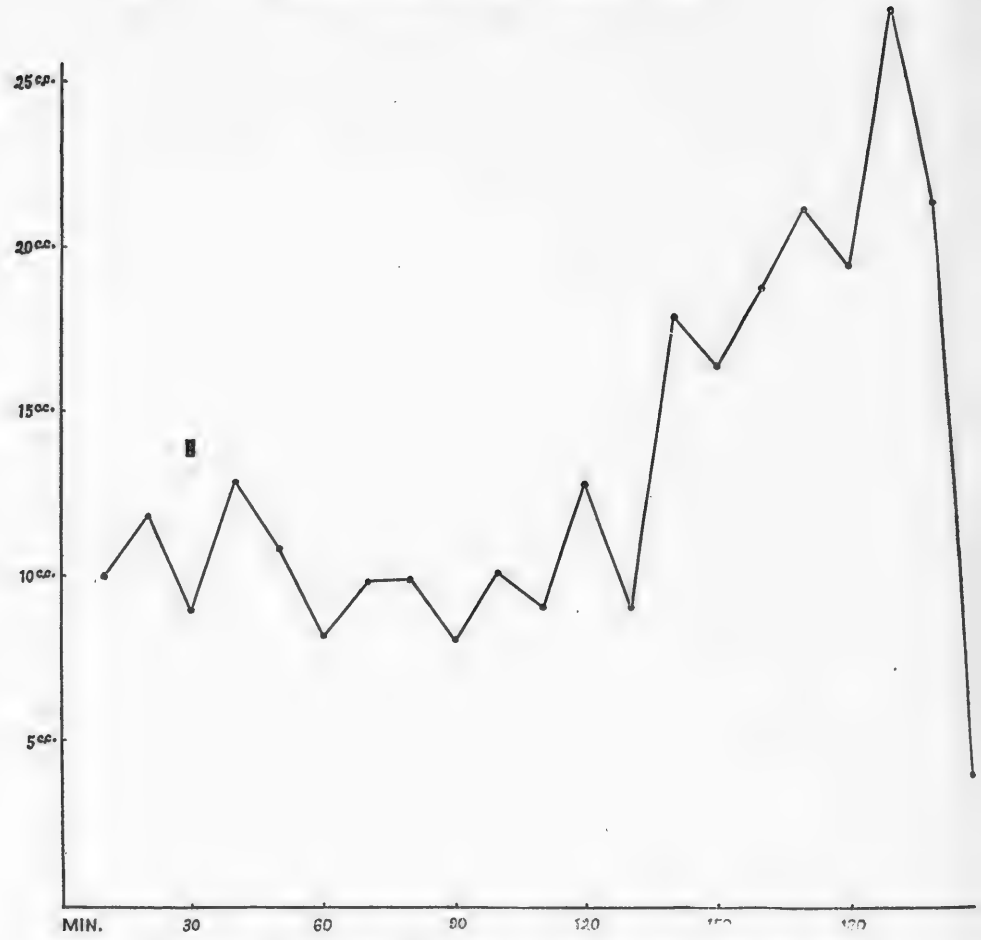
In the following experiment, injection of cantharidin, as in experiments 4 and 5, caused a well marked increase in the flow of lymph, but this increased flow was not preceded by a period during which the flow was diminished.

Experiment 6.—Dog, weighing 18,600 gm. A cannula was placed in the thoracic duct (text-figure 4).

Time.	Lymph in c.c. per 10 min.	Remarks.
12:10–12:20 P. M.	10.0	Lymph opaque, white, slightly blood tinged.
12:20–12:30 P. M.	11.8	
12:30–12:40 P. M.	8.9	At 12:40 cantharidin, 0.02 gm., intramuscularly.
12:40–12:50 P. M.	12.8	
12:50– 1:00 P. M.	10.7	
1:00– 1:10 P. M.	8.3	
1:10– 1:20 P. M.	9.8	
1:20– 1:30 P. M.	9.8	
1:30– 1:40 P. M.	8.2	
1:40– 1:50 P. M.	10.3	Lymph blood stained.
1:50– 2:00 P. M.	9.2	
2:00– 2:10 P. M.	12.9	
2:10– 2:20 P. M.	9.2	
2:20– 2:30 P. M.	18.0	
2:30– 2:40 P. M.	16.5	
2:40– 2:50 P. M.	18.8	
2:50– 3:00 P. M.	21.3	
3:00– 3:10 P. M.	19.5	
3:10– 3:20 P. M.	27.3	
3:20– 3:30 P. M.	21.4	
3:30– 3:35 P. M.	4.0	Died at 3:35.

Autopsy.—The liver is moderately deep red. There is no edema of the gall bladder. The regional lymphatic nodes are enlarged and have red centers. Dilated lymphatics pass from the liver to these nodes.

The experiments which have been described are confirmed by others in which similar results have been obtained. The effect of cantharidin upon the flow of lymph from the thoracic duct is as follows: (a) Intramuscular injection of a fatal dose (0.02 of a gram) is usually followed by well marked diminution of the flow, which is evident after from twenty to thirty minutes and reaches a minimum at the end of about one hour; the lymph is tinged with blood. No subsequent rise occurs and there is edema of the liver recognizable within from two to three hours after injection. (b) In a second group of experiments occurring more frequently with larger doses of cantharidin (0.05 of a gram), an increased flow of lymph follows the retardation which has been noted or occasionally occurs without a preceding diminution of output. This increased



TEXT-FIG. 4. Experiment 6, showing the flow of lymph from the thoracic duct after injection of cantharidin.

flow, occurring after an interval approximately from one and a half to two hours after injection of the drug, may greatly exceed the normal. The lymph is no longer tinged with blood but assumes a yellow color and coagulates slowly.

Within the period of observation permitted by the experiment larger doses more frequently demonstrate the lymphagogue action

of the poison. With smaller doses the flow of lymph does not recover from its primary depression. Dilatation of lymphatics and edema of the liver and gall bladder occur during the time when flow of lymph from the thoracic duct is diminished and is almost constantly observed when death is caused by acute poisoning with cantharidin. Edema of the liver furnishes evidence that there is increased lymph formation in the liver before this increased production causes an increased flow from the thoracic duct. Histological examination shows that obstruction to the lymph stream capable of producing simultaneously edema and diminished flow of thoracic lymph occurs within lymphatic channels and notably within the sinuses of the regional nodes of the liver.

Those nodes which receive lymph from the liver are accurately defined by the poison. Their situation has already been described; they become enlarged and their medulla assumes a deep red color. The nodes which are affected are connected with the liver by lines of dilated lymphatics which pass downward from liver and gall bladder to the proximal nodes and hence from one node to the next. These lymphatics contain lymph deeply tinged with blood and their termination in the peripheral sinus of the node is well defined by similar localized discoloration.

Microscopic examination of the regional nodes removed from animals which have died with edema of the liver and gall bladder during the period of diminished flow from the thoracic duct shows that the afferent lymphatics and the sinuses of the node, notably the peripheral sinus, contain fibrin which usually forms a fine network but occasionally occurs in more compact particles. Particularly noteworthy is the relationship of this fibrinous network to cells which have undergone degenerative changes. Cells of which the nuclei are pale and ill defined or wholly lost form centers from which radiate fibrils of fibrin. A similar radiate arrangement of fibrin occurs upon the free surface of endothelial cells still attached to the walls of the sinus.

Within the widely dilated sinuses of the node, cells are very numerous, often closely packed together and connected by the fibrinous network which has been described. Large mononuclear cells similar to those which line the sinuses are particularly numer-

ous and are mingled with lymphoid cells and polynuclear leucocytes, the latter being present in moderate number. Cells exhibiting evidence of degeneration and necrosis, doubtless caused by the poison, are numerous; many exhibit nuclear fragmentation; others have assumed a hyaline appearance and have lost their nuclei. The destructive action of cantharidin is best seen in the germinating centers of the cortex where nuclear fragmentation is usually widespread. The presence of red blood corpuscles outside the capillaries of the liver, within the hepatic lymphatics and in the sinuses of the lymphatic nodes, is further evidence of the injurious action of the poison which appears to affect notably endothelium of the blood-vessels and lymphatics. Phagocytosis of red blood corpuscles by the large mononuclear cells which have been mentioned is actively in progress two to three hours after administration of cantharidin. Clumping of red blood corpuscles upon the surface of these cells may have a part in plugging the sinuses of the regional hepatic nodes. In one specimen masses of red corpuscles were adherent to the surface of mononuclear phagocytes which had taken many red corpuscles into their substance. Such observations show that the cell does not move toward the red corpuscles and ingest it, but attracts the corpuscle and holds it. I have not determined whether the conspicuous yellow color assumed by the lymph from the thoracic duct is referable to destruction of red corpuscles with setting free of hemoglobin or to the presence of bile liberated by the injured liver.

Progressive dilatation of lymphatics and of the sinuses of the lymphatic nodes finally overcomes the obstruction opposed by the fibrinous thrombi which are formed in these channels. Diminished coagulability of the lymph probably explains the cessation of progressive formation of fibrinous plugs. Increased flow of lymph often far exceeding the normal thus follows the temporarily diminished output.

In order to determine the relationship of lymphatic nodes to the changes which occur in the liver, the effect of cantharidin has been tested upon animals from which the regional hepatic nodes have been extirpated. Removal of these nodes may be followed by establishment of collateral lymphatic circulation through channels which

pass directly to the thoracic duct without intervention of lymphatic nodes. Nodes which have been overlooked at the time of operation undergo hypertrophy. Whereas in normal animals fatal cantharidin poisoning has been almost invariably followed by edema of the liver and gall bladder, edema has occurred in only one of three animals in which, after extirpation of the regional nodes, death has followed administration of the drug. In this instance there was edema of the liver, gall bladder, and pancreas, and accumulation of fluid in the peritoneal cavity; a large lymphatic node was found in the gastrohepatic omentum and numerous dilated lymphatics passed to it from the liver. This enlarged node had undergone the changes which have been described, the sinuses being plugged with fibrin.

Experiment 7.—Dog, weighing 9,800 gm. The abdomen was opened and all the lymphatic nodes which could be found in the gastrohepatic omentum, in the duodenal mesentery and near the splenic vein along the upper margin of the splenic arm of the pancreas were removed. On the 22d day after operation, cantharidin, 0.013 gm., was administered intramuscularly; on the following day, 724 c.c. of urine contained 4.16 gm. of fermentable sugar and albumin in abundance; on the next day sugar was still present. On the 26th day cantharidin, 0.006 gm., was administered; 550 c.c. of urine collected during the following day contained 2.43 gm. of sugar and abundant albumin. On the 30th day cantharidin, 0.013 gm., was administered and albuminuria but no glycosuria followed. On the 40th day cantharidin, 0.02 gm., was given; death occurred during the following night. The urine, 330 c.c., contained 3.52 gm. of sugar (Benedict's method), and albumin, determined by Esbach's method, equal to 2.667 gm. per liter.

Autopsy.—The peritoneal cavity contains 23 c.c. of red serous fluid. The liver is deep red; the gall bladder is edematous throughout, the wall measuring 2 to 3 mm. in thickness. Many dilated lymphatics filled with blood stained lymph pass in parallel lines to a firm, deep red lymphatic node 2 cm. in length, situated in the gastrohepatic omentum at the margin of the foramen of Winslow. Two smaller blood tinged nodes are found at the base of the duodenal mesentery. The tissues of the gastrohepatic omentum are edematous. The heart is normal save for edema of the fibrous tissue at the base of two aortic segments accompanied by edema of the segment of the tricuspid valve on the opposite side of the interventricular septum. The thymus is dull red throughout; the adjacent substernal lymphatic nodes are enlarged and red and the tissue about them is edematous.

Microscopical examination shows well marked edema of the liver and pancreas. In many places hepatic cells have separated from one another so that they are no longer arranged in columns. The cells composing acini and islands of Langerhans of the pancreas are similarly separated. The sinuses of lymphatic nodes in the positions which have been mentioned contain fibrin in abundance. Here fibrin often radiates from the surface of necrotic cells.

In the following experiments no large lymphatic nodes escaped the operation planned for their removal, and edema of the liver and gall bladder failed to occur.

Experiment 8.—Dog, weighing 8,000 gm. The operation previously described was repeated. On the 22d day after operation, the animal had gained 1,200 gm. in weight and received cantharidin, 0.013 gm.; 870 c.c. of urine passed during the following day contained 4.61 gm. of sugar. Cantharidin, 0.006 gm., on the 25th day caused no glycosuria. Death followed within 15 hours after the administration of cantharidin, 0.02 gm.; 925 c.c. of urine contained fairly abundant albumin but no sugar.

Autopsy.—The liver is deep red; there is no edema of the gall bladder. Above the splenic arm of the pancreas is a small lymphatic node 0.3 by 0.5 cm. The pancreas is slightly edematous and there is well marked edema of the gastrohepatic omentum in the neighborhood of the lesser curvature of the stomach. Microscopical examination of the liver shows congestion but no edema.

Experiment 9.—Dog, weighing 6,000 gm. The regional hepatic nodes together with other abdominal lymphatic nodes were removed. Cantharidin, 0.013 gm., administered on the 18th day caused death after 40 hours; urine, 820 c.c., collected during the following day contained abundant albumin and 8.04 gm. of sugar (Benedict's method; fermentation not tested), and on the next day the urine (80 c.c.) contained a moderate amount of albumin and 0.18 gm. of sugar (fermentation not tested).

Autopsy.—The liver is pale brownish red; there is no edema of the gall bladder. Dilated lymphatics pass from the liver and uniting form a large trunk which passes into the retroperitoneal tissue and joins the receptaculum chyli. No lymphatic nodes are found at the base of the duodenal mesentery or along the superior margin of the splenic arm of the pancreas. Microscopical examination shows no edema of the liver or pancreas.

The experiments are inconclusive with regard to the effect of the removal of lymphatic nodes upon the action of cantharidin. When, on the one hand, some of the regional nodes escape operative removal (experiment 7), these nodes under the influence of cantharidin may oppose obstruction to the flow of lymph and in consequence edema occurs. When, on the other hand, all nodes are removed, collateral circulation (experiment 9) may permit the lymph to flow into the thoracic duct and no edema follows the administration of cantharidin. Although the number of experiments is too small to define the action of cantharidin in animals deprived of regional hepatic lymphatic nodes, they support the view that occlusion of lymphatic channels, in association with increased lymph formation, is the cause of the edema which follows administration of cantharidin.

The foregoing experiments define the changes which accompany

edema of the liver and gall bladder. Separation of columns of liver cells from adjacent capillaries, the intervening space containing serum and often red blood corpuscles, and separation of liver cells from one another, are manifestations of edema of the liver.

The flow of lymph from the thoracic duct is profoundly modified by cantharidin; after administration of the drug, the flow diminishes or ceases completely. Coincident with cessation and within three hours after administration of the poison, edema of the liver and gall bladder may be well advanced. Edema caused by cantharidin is almost wholly limited to these organs and to the adjacent lymphatic nodes.

Edema of the liver is dependent upon changes in the lymphatics and in the lymph sinuses of the regional lymphatic nodes, the effective change being the formation of fibrinous coagulum within the sinuses. This coagulum often has its origin about cells injured by the poison, shreds of fibrin radiating from partially or completely necrotic cells.

Obstruction interposed by fibrin formations within lymphatic nodes is overcome in a certain number of experiments, and diminished flow from the thoracic duct is followed by an output which may considerably exceed that observed before administration of the poison. Distension of lymphatics in the liver and occurrence of edema during the period of diminished flow indicates that increased formation of lymph occurs in the liver before increased flow of thoracic lymph demonstrates its presence. In one experiment increased flow was not preceded by a period of diminished output; obstruction to outflow from lymphatics failed to occur or was insufficient to diminish the flow of lymph below normal. In this experiment edema of the gall bladder and liver was absent.

A poison which is capable of causing nephritis increases the flow of lymph from the thoracic duct; it acts as a lymphagogue. In the presence of some additional factor, such as obstruction within the regional lymphatic nodes, edema results. These changes are coincident with the production of the renal lesion and are not the result of disturbance of renal function. They furnish, by analogy, support to the view that dropsy in association with nephritis is caused by the action of an irritant which simultaneously injures the kidney and increases the permeability of vessel walls elsewhere in the body.

EXPERIMENTS ON INSECT TRANSMISSION OF THE VIRUS OF POLIOMYELITIS.*

By C. W. HOWARD and PAUL F. CLARK.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

In considering the epidemiology of epidemic poliomyelitis we are confronted with a complex problem. Not only do we find cases which can be traced either through direct or indirect contact to other persons ill with the disease, but also we meet sporadic cases occurring over widely scattered areas and apparently well isolated from any acute source of contagion.

Both in human and in experimental poliomyelitis the virus has been repeatedly demonstrated in the tonsils and the secretions of the nasopharyngeal mucosa.[1] As the experimental disease can also be readily produced by swabbing the nasal mucosa with the virus, it has been held by many that the nasal mucosa is one, at least, of the sources of the virus in the outside world and also the means of its entrance to the body. The marked viability of the virus under adverse conditions, such as drying, low temperature, etc., and the fact demonstrated by Neustaedter and Thro[2] that the virus remains alive in dust, must also be considered as making for a fairly adequate explanation of the occurrence of cases in neighboring localities. More recently Kling, Wernstedt, and Pettersson[3] have brought forward experimental evidence that the nasal secretions may also be an important factor in the sporadic cases, as they claim to have found the virus in nasal washings from persons in contact with acute cases, as well as from the cases themselves.

* Received for publication, October 10, 1912.

[1] Flexner, Simon, and Lewis, Paul A., *Jour. Am. Med. Assn.*, 1910, liv, 535; Osgood, R. B., and Lucas, W. P., *idem*, 1911, lvi, 495; Landsteiner, K., Levaditi, C., and Pastia, C., *Compt. rend. Acad. d. sc.*, 1911, clii, 1701.

[2] Neustaedter, M., and Thro, W. C., *N. Y. Med. Jour.*, 1911, xciv, 813.

[3] Kling, C., Wernstedt, W., and Pettersson, A., *Ztschr. f. Immunitätsforsch., Orig.*, 1911–12, xii, 316, 657; 1912, xiv, 303.

The fact that the seasonal incidence of the disease corresponds in general with the annual wave of insect life still suggests, however, the possibility that some insect may play a part in the transmission of this disease.

We have carried out experiments with several insects that are in close relation to human beings with reference to their ability to carry the poliomyelitic virus. Two types of insects come at once into consideration. One inhabits man's surroundings and acts as a mere contaminator of objects with which he is in intimate relation. These insects do not bite and are not to be viewed as possible active inoculators of the virus. An example of this group is the common house fly, *Musca domestica.* The other type comprises some of the biting insects. Among these are species that possess limited means of locomotion, such as the bedbug (*Cimex lectularius*) and lice (*Pediculus capitis* and *Pediculus vestimenti*), and others, such as the different mosquitos which have comparatively a much greater power of locomotion. These may conceivably act as active inoculators.

The domestic fly is by reason of its habits readily subject to gross contamination provided it can find access to infectious material. The existence of the poliomyelitic virus in the secretions of the nose and throat and also in the discharges from the intestine (Kling, Wernstedt, and Pettersson,[4] and Flexner, Clark, and Dochez[5]) makes such access possible. Whether the contamination ever occurs in nature is not known.

Our experiments were, in general, performed in two ways. In the first, insects were permitted to feed directly on infected cord or upon materials containing a suspension of infected cord. After the removal of the infected materials, the insects were placed in a fresh receptacle and after varying periods of time were killed and used whole or in part for the preparation of bacteria-free Berkefeld filtrates that were inoculated into monkeys. In this way we sought to determine whether the poliomyelitic virus survived either within the bodies of the insects or upon their external parts. In the

[4] Kling, C., Wernstedt, W., and Pettersson, A., *loc. cit.*

[5] Flexner, Simon, Clark, Paul F., and Dochez, A. R., *Jour. Am. Med. Assn.*, 1912, lix, 273.

second group, biting insects were made to feed upon monkeys inoculated with the virus of poliomyelitis. It has been shown by Flexner and Lewis[6] that the virus is present in the blood stream of infected monkeys, but only in rather minute quantities. The dilution is so great that twenty cubic centimeters or more of the blood from a recently paralyzed monkey are usually necessary to produce the disease in a normal animal. On account of this high dilution, it is especially valuable to determine whether the virus may be taken up by blood-sucking insects in sufficient quantity to produce infection when a filtrate of their bodies is employed for inoculating healthy monkeys. This experiment was carried out with bedbugs and lice.

EXPERIMENTS WITH FLIES.[7]

The flies used were the ordinary species, *Musca domestica,* bred in the laboratory. The technique of the experiment has been described above. After being allowed to feed on infected cord, the flies were placed in fresh receptacles from which certain numbers were removed at intervals, killed with ether, ground up with sand and physiological salt solution, and passed through a Berkefeld filter. The filtrate was injected as usual into the brain of *Macacus rhesus* monkeys. Protocols A and B show that the flies retained the virus either in or on their bodies for at least twenty-four and forty-eight hours respectively.

In order to determine the presence or absence of the virus actually within the bodies of the flies, the viscera of certain flies that had fed on infected cord were removed with aseptic precautions. Protocol C gives the history of such an experiment proving that the virus may remain alive in the body of the fly for at least six hours after ingestion.

Protocol A.—May 2, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 3 c.c. of filtrate from the bodies of 7 flies which had had an opportunity to feed on infected cord for 5 hours and had then lived in clean surroundings for 24 hours before they were killed. The monkey recovered from the ether anesthesia and was apparently normal.

May 8. The monkey shows slight weakness.

[6] Flexner, Simon, and Lewis, Paul A., *Jour. Exper. Med.,* 1910, xii, 227.

[7] Flexner, Simon, and Clark, Paul F., *Jour. Am. Med. Assn.,* 1911, lvi, 1717.

May 9. Animal is nervous and irritable. Right arm is weak, left arm definitely paralyzed. During the day the paralysis affected the right arm.

May 10. The monkey is completely prostrate. The animal was killed with ether. At autopsy the cord showed the characteristic macroscopic lesions of experimental poliomyelitis. The microscopic sections were also typical.

Protocol B.—May 3, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 4 c.c. of filtrate from the bodies of 10 flies which had fed on infected cord 48 hours previously. The monkey was apparently normal after recovery from ether anesthesia and remained well until May 13.

May 14. Animal is completely prostrate with paralysis of back muscles and all four extremities.

May 15. Animal was killed with ether. The autopsy showed characteristic lesions of experimental poliomyelitis in the spinal cord, and the sections, typical histological lesions.

Protocol C.—May 17, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 5 c.c. of filtrate representing the viscera of 9 flies which had had an opportunity to feed on infected cord for about 18 hours. They were then removed to a clean receptacle for 6 hours, when they were etherized, and their legs, wings, and heads removed. The viscera were then taken out with sterile instruments, ground and filtered as usual, and injected as above. The monkey carries its head towards the left side after recovery from ether and seems somewhat dazed.

May 18. The monkey is active and apparently normal.

May 29. The animal shows a definite tremor and excitability and has the characteristic worried expression. The coat is fluffed up. Cerebrospinal fluid shows marked lymphocytosis and gives a positive test for globulin. 5.00 P. M. Arms very weak. Tremor is more marked. Animal gets up on perch with great difficulty.

May 30. Animal is prostrate. Legs and back muscles are partly but definitely paralyzed. Arms are completely flaccid.

May 31. Animal is moribund. Killed with ether. At autopsy the gross lesions in the cord were characteristic and the microscopic sections were also typical.

EXPERIMENTS WITH MOSQUITOS.

The mosquito experiments were performed in much the same way as were those with flies, except that the sticky, viscid nature of the incised cord caught the mosquitos as a piece of sticky fly-paper would, so a variation in the method of bringing them in contact with the virus was adopted. A mixture of defibrinated blood and a heavy suspension of cord from a recently paralyzed monkey was injected into washed figs, and these figs were suspended in the jars with the mosquitos. In this way the mosquitos apparently took the blood-virus mixture very well. After being fed on this

mixture for two days the mosquitos were placed in clean jars with non-injected figs and water and kept alive until wanted.

In two experiments the mosquitos used were the common *Culex pipiens.* In all the others the mosquitos were the salt marsh varieties, about 90 per cent. in each lot being *Culex sollicitans* and 10 per cent. *Culex cantator.*

Numbers of them were removed at intervals varying from three hours to twenty days after they had fed on the virus mixture. They were then killed, ground up, passed through a Berkefeld filter, and injected into the brain of a *Macacus rhesus* monkey. The purpose in allowing longer periods to elapse after the mosquitos had fed on the virus was to determine if possible whether there might be some biological transformation of the virus in the body of the mosquito as a secondary host. Fifteen monkeys were injected with mosquitos removed at different intervals and not one of these monkeys showed any symptoms of poliomyelitis. A control monkey inoculated with a minute portion of a fig injected with the blood-virus mixture came down typically with poliomyelitis.

Two illustrative protocols follow, showing the negative nature of the results, and also the protocol of the control animal (protocols D, E, and F).

Protocol D.—Aug. 12, 1911. Injected intracerebrally into a *Macacus rhesus* monkey 3.5 c.c. of filtrate representing the bodies of 20 mosquitos which had fed on the blood-virus mixture 24 hours previously. The animal remained normal and active.

Protocol E.—Sept. 16, 1911. Injected into the brain of a *Macacus rhesus* monkey 4 c.c. of filtrate representing the bodies of twelve mosquitos that had had an opportunity to feed on active poliomyelitis virus in figs for 2 days and had lived in clean surroundings for 20 days thereafter. The wings and legs were removed, the mosquitos ground up, filtered and injected as above. The monkey showed a slight weakness on the day following the injection but no other symptoms or paralysis.

Protocol F.—(Control of blood-virus mixture in figs.)

June 1, 1912. Injected into the brain of a *Macacus rhesus* monkey 3 c.c. of the following filtrate: On May 29 about 5 c.c. of a blood-virus mixture (2.5 c.c. of defibrinated horse blood and 2.5 c.c. of a 5 per cent. suspension of the cord from a recently paralyzed monkey) were injected into a fig weighing about 20 grams and allowed to remain at room temperature until June 1. A 5 per cent. suspension was then made of this fig and the material shaken, centrifugalized, and filtered. The filtrate was injected as above, thus introducing into the monkey filtrate which originally contained approximately 0.013 gm. of the cord.

June 6. Monkey somewhat inactive.

June 7. Slightly excitable. Arms and legs are weak, especially arms.

June 8. The arms, legs, and back muscles are paralyzed.

June 9. Found dead in the morning. At autopsy there were slight characteristic lesions in the cord and the microscopic sections were typical for experimental poliomyelitis.

EXPERIMENTS WITH LICE.

The experiments with *Pediculus vestimenti* and *Pediculus capitis* belong in the second general group; that is, the insects were allowed to suck the blood of several monkeys infected with epidemic poliomyelitis and then to remain alive for a number of days before they were killed, ground up in salt solution, filtered, and injected into monkeys. These insects proved to be difficult to manipulate, the mortality among them being very high. After their last feeding upon an infected monkey, it was found impossible to keep them alive for more than two to four days even though they were fed daily on a normal monkey. In only one experiment were we able to keep sufficient numbers of *Pediculus capitis* alive for as long as two days after their last feeding. The *Pediculi vestimenti* were used in five experiments in which they received from one to six feedings on monkeys with poliomyelitis, some in the preparalytic stage and others definitely paralyzed. The feedings were usually given either every day or on alternate days, so that in no case did any of the insects remain alive for more than thirteen days after the first feeding.

The results were entirely negative. The following are two illustrative protocols (protocols G and H).

Protocol G.—Dec. 19, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 3 c.c. and intraperitoneally with 5 c.c. of filtrate representing in all 24 *Pediculi capitis* that had fed on a poliomyelitic monkey from Dec. 7 to Dec. 18. They were then made into a filtrate and injected as above on Dec. 19. The monkey showed no untoward symptoms or paralysis.

Protocol H.—Jan. 17, 1912. Injected a *Macacus rhesus* monkey intracerebrally with 2.25 c.c. of filtrate and intraperitoneally with 0.5 c.c. of filtrate representing 13 body lice which had fed on paralyzed monkeys from Jan. 9 to Jan. 13. On Jan. 15, the lice were fed on a normal monkey and on Jan. 16 they were ground up and passed through a Berkefeld filter. The monkey remained active and well.

EXPERIMENTS WITH BEDBUGS.

The experiments with bedbugs (*Cimex lectularius*) also belong to the second group and were carried out as were those with the lice, except that the greater ease of manipulation made it possible to perform a more complete series.

The bedbugs were in all cases fed at least six times on infected monkeys in various stages of the experimental disease. More of the feedings were made on monkeys in the preparalytic stage than on those in a paralyzed condition because of the probably greater concentration of the virus in the blood stream during that period. It was found impossible to induce the bedbugs to feed until the previous meal had been digested, and, in the case of the immature forms, until the skin had been molted. Hence four or five days to a week passed between the successive feedings. After the last feeding on infected monkeys, periods ranging from a few hours to twenty-five days were allowed to elapse before the insects were ground up in salt solution and filtered preparatory to injection into a normal monkey. As in the case of the other insects this period between possible ingestion of the virus and the killing of the bedbugs was designed to answer the question of a possible life cycle in the body of the insect. Since many of the bedbugs were fed more than six times, some of them remained alive from seven to nine weeks after their first feeding.

In all, sixteen monkeys were injected with filtrates from the bodies of these bedbugs, and of this number only one showed evidence of infection with the virus of epidemic poliomyelitis. A repetition of the successful experiment, however, with the same time limits, etc., proved unsuccessful.

Two illustrative protocols are given.

Protocol I.—Dec. 18, 1911. Injected into the brain of a *Macacus rhesus* monkey 3 c.c. of filtrate, and intraperitoneally 7 c.c. of filtrate representing in all 20 bedbugs that had fed on poliomyelitic monkeys on the following dates: Nov. 8, 13, 15, 20, 29, and Dec. 5.

The filtrate was made on Dec. 15; thus an interval of 10 days occurred between the last feeding and the killing of the insects. The monkey showed no symptoms or paralysis.

Protocol J.—Nov. 24, 1911. Injected intracerebrally a *Macacus rhesus* with 2.5 c.c. of filtrate representing 10 bedbugs fed on infected monkeys on the

following dates: Oct. 23, 24, 27, Nov. 3, 8, 13, and 17. On Nov. 24, 7 days after the last feeding, the bedbugs were killed, filtered, and injected as above.

Nov. 30. Monkey somewhat slow. Arms are weak.

Dec. 1. Arms are partly paralyzed. The back and legs seem weak. Animal lies down most of the time.

Dec. 2. Animal moribund. Killed with ether. At autopsy the animal was found to have a severe tuberculosis of the spleen and left lung. The liver, mesenteric nodes, and right lung were also involved, but to a less degree. The right lung had only three or four tubercles. There were no definite macroscopic lesions in the cord. The microscopic sections, however, showed the typical lesions of experimental poliomyelitis.

A suspension of the glycerinated cord from this monkey was inoculated into another animal and this second animal came down typically, developing a definite paralysis on the sixth day. This animal was killed with ether. At the autopsy marked characteristic lesions were evident in the cord and the microscopic sections confirmed the result. The histological alterations in both spinal cords were characteristic of experimental poliomyelitis in the monkey and showed both infiltrative lesions about the blood-vessels in the interstitial substance and also degeneration and necrosis of ganglion cells and neurophagocytosis.

During the series of feeding experiments both with the lice and the bedbugs, the insects were sometimes allowed to feed on normal monkeys when no infected monkeys happened to be available at the time a feeding was necessary. Also during the varying intervals occurring after the last feeding on infected animals, normal animals were used in order to keep the insects alive. While this was not a definite attempt to study direct transmission by means of these insects, it is worthy of note that none of these normal animals so used ever showed the least symptom of poliomyelitis.

DISCUSSION.

The deductions from these experiments are clear. The domestic fly may become contaminated with the poliomyelitic virus which it may obtain in nature from infected discharges from the nose and throat or intestine. Because of the resistance of the virus to ordinary physical changes, especially when combined with mucous secretions, it can be transported in a living state on the surface of their bodies for two or more days, and because of the resistance it

displays to digestive secretions, within the esophagus and stomach for at least several hours. The survival in the gullet permits of the contamination of objects during the feeding process through the act of regurgitation which the fly performs in moistening and dissolving its food.

The flight of the house fly is considerable. Copeman, Howlett, and Merriman[8] found marked flies from one half to three quarters of a mile from the place of their liberation. On this account these potential passive contaminators are theoretically capable of carrying and depositing the virus at a considerable distance from the point at which the original contamination occurred. Through the ordinary active habits of the fly the virus may be transferred to persons or to things with which persons come into close relation, and by the death of the flies they may through disintegration liberate surviving virus that may possibly be converted into dust. The finding of the virus in the secretions of the nose, throat, and gastro-intestinal tract of human beings suggests that through the agency of such a passive contaminator as the fly, the virus may be taken into the bodies of human beings in which, conditions favoring, it may develop and cause infection.

Our experiments tend to exclude at least the species of mosquitos with which we worked as carriers of the virus. The pediculi also seem incapable of taking the virus out of the blood or of maintaining it in a living state within their bodies.

The experiments with the bedbug have a special interest. They were not designed either to ask or answer directly the question whether the bedbug acts as the source of infection in man. The question asked was whether they (or other biting insects) could take up the virus from the blood and maintain it for a period in a living state. When it is recalled that the virus exists in a highly dilute condition in the blood, this question assumes considerable importance. Although only a single successful experiment was secured, it is none the less clear in its meaning, which is that it is possible for a blood-sucking insect both to obtain living virus from the blood and to maintain it in a living state for at least seven days,

[8] Copeman, S. M., Howlett, F. M., and Merriman, G., *Reports of the Local Government Board on Public Health and Medical Subjects,* 1911, No. 53, 1.

at which time it can be inoculated successfully into the monkey and experimental poliomyelitis produced. Since, however, the experiment succeeds so exceptionally, the bedbug would seem not to be a carrier of the poliomyelitic virus in nature, although this possibility is not entirely excluded.

CONCLUSIONS.

The domestic fly (*Musca domestica*) can carry the virus of poliomyelitis in an active state for several days upon the surface of the body and for several hours within the gastro-intestinal tract.

Mosquitos (*Culex pipiens, Culex sollicitans,* and *Culex cantator*), in our experiments, have not taken up and maintained in a living state the virus from the spinal cord of monkeys.

Lice (*Pediculus capitis* and *Pediculus vestimenti*) have not taken the virus out of the blood of monkeys or maintained it in a living state.

The bedbug (*Cimex lectularius*) has taken the virus with the blood from infected monkeys and maintained it in a living state within the body for a period of seven days.

STUDIES ON FERMENT ACTION.

V. Immunization with Proteolytic Cleavage Products of Pneumococci.*

By JAMES W. JOBLING, M.D., and SOLOMON STROUSE, M.D.

(*From the Morris Institute for Medical Research, Chicago.*)

Experimental studies of the pneumonic process, based on the conceptions of modern biological science and couched in commonly adopted terms of immunity, have as yet not succeeded in explaining the disease. Wadsworth (1) has recently given a thorough presentation of the status of biological studies on pneumonia and has shown how unsatisfactory our knowledge is. Perhaps of the commoner forms of immune bodies the opsonins have played the most important part in the literature of this problem, yet phagocytosis has been shown to be not the only, or even necessarily the most important factor. Our own studies on opsonins (2) in pneumonia led us to believe that whereas they were present in the circulating blood of pneumonia patients after crisis, they could not *per se* explain the crisis. Yet phagocytosis seems to have thrown the most light on the subject of immunity in pneumonia (Lamar (3)).

If, then, the phenomena of pneumonia have not yielded to the probings of biological investigation, it is necessary to adopt new or hitherto unstudied lines of work. Lamar (3) reasoning in a similar vein directed his attention to the effects on pneumococci of various soaps and found an interesting correlation between the effects of soaps and immune sera. Rosenow (4, 5, 6) studied mainly the separation of the toxic substances in the body of the pneumococcus and the relation of these substances to proteolysis. Dick (7) demonstrated the presence in post-critical pneumonic sera of a proteolytic ferment capable of a specific digestion of pneumococcus extracts. All these investigations will be discussed more fully in the course of this and subsequent papers. They are men-

* Received for publication, August 22, 1912.

tioned here in order to emphasize various new points of attack of the problem.

For the past two years we have been focussing our attention on the problem from the view-point of possible ferment action, and we have been experimenting with several of the body ferments. Along with other comparative studies we presumed that the high circulating leucocytosis and especially the accumulation of leucocytes in the lung must play an important part in the cure of pneumonia. More than theoretical support is lent to this view by the fact that pneumococcus infections other than pneumonia are not attended by the same clinical picture. Neither general pneumococcus septicemias nor localized infections such as arthritis or endocarditis, which are not associated with local accumulations of leucocytes, terminate by crisis. The conclusion that the leucocytes play a part in the immunity process in pneumonia is, of course, not proved, but the association is certainly suggested. Since phagocytosis cannot explain the crisis, it is necessary to consider the other possible activities of leucocytes.

In a previous paper (8) we reported our investigations on some of the leucocytic ferments causing proteolysis. In this paper we were able to show the presence of at least three such ferments, two capable of splitting native protein, and one acting on the products of digestion by proteases. By the process of drying with alcohol and ether, by the effect of heating, and by the use of different zymolites in acid or alkaline media, we were able to separate the actions of these various ferments. We showed that the alkaline extraction of leucocytes which had been dried by means of alcohol and ether yielded a ferment capable of splitting native protein to albumose and to a slight extent to peptone, whereas fresh untreated leucocytes were practically identical with trypsin in their proteolytic activities.

In the present paper we propose to discuss (1) the effect of the alkali-acting leucoprotease on pneumococci and (2) experiments dealing with the immunization of animals by the split products of this digestion.

In a series of experiments the comparative effects of physiological salt solution and of leucoprotease on pneumococci were tested.

These experiments involved simple technique. Given amounts of twenty-four hour broth culture pneumococci were centrifugalized, washed in physiological salt solution, resuspended in salt solution, and divided into two equal parts. To one half was added a small amount of the alkaline leucoprotease, to the other the same amount of salt solution. A few drops of chloroform[1] were added and the tubes incubated at 37° C. Every day the gross appearance of the tube and the microscopic changes in the organisms were noted. Marked differences in the gross appearance of the flasks could be seen in twenty-four hours. The turbidity of the flask containing leucoprotease was greatly diminished, whereas the flask containing autolyzing pneumococci was only slightly less cloudy than when they were put in the incubator. Usually within forty-eight hours the leucoprotease flask had almost entirely cleared up, leaving a minimum deposit, but the salt flask was still cloudy. If the digestion were continued as long as one week the differences between the two were still marked. In no instance did the flask containing autolyzing pneumococci show as rapid or as complete clearing as did the one containing leucoprotease and pneumococci. As might be expected, the microscopic appearances of the pneumococci were parallel to the gross changes. The leucoprotease flask showed a much more rapid diminution in the number of organisms, and tinctorial changes evidencing disintegration of those remaining. Even within twenty-four hours most of the pneumococci were mere shadows and were Gram negative, whereas in the salt flasks the organisms looked almost normal and were still Gram positive. At the same time, the chemical changes in the two tubes were compared. The method of measuring the various proteolytic fractions evolved in our previous studies could not be carried on in these experiments, as such technique was only possible with larger amounts of zymolite. Certain constant changes, however, were found, which indicate that the organisms treated with leucoprotease underwent deeper proteolysis in a shorter time than did those subjected to autolysis in salt solution. The pneumococci treated with leucoprotease constantly gave a higher figure for total acidity, as

[1] We have found that pneumococci contain an antiproteolytic substance, the action of which is prevented by chloroform. This will be discussed more fully in a subsequent paper.

measured by titration with N/50 sodium hydroxide, and gave a figure with formol titration which was constantly higher than that obtained from the salt control. The results here were always the same, whether we used dried pneumococci or fresh washed ones, or whether the salt control was rendered to the exact alkalinity of the leucoprotease tube. Although we realize the limitations of the formol titration method of measuring proteolysis, the constancy of our results warrants its application.

When we studied the chemical changes from day to day it was seen that leucoprotease produced its maximum digestion in from twenty-four to forty-eight hours, and that after that time there was a decline in both the total acidity and the figure for formol titration. In table I are given the results of one such experiment, showing also in a parallel column the figures for the autolyzing pneumococci. In this experiment the pneumococci in salt solution gave no evidence of digestion as measured by formol titration, but in other experiments slight digestion could be ascertained.

TABLE I.

Contents of flask.	Incubation period.	Acidity in c.c. of N/50 sodium hydroxide.	Formol titration in c.c. of N/50 sodium hydroxide.
Pneumococci and leucoprotease.	24 hrs.	0.20	0.37
Pneumococci in salt solution.	24 hrs.	0.11	0.00
Pneumococci and leucoprotease.	48 hrs.	0.23	0.24
Pneumococci in salt solution.	48 hrs.	0.17	0.00
Pneumococci and leucoprotease.	96 hrs.	0.09	0.12
Pneumococci in salt solution.	96 hrs.	0.06	0.00
Pneumococci and leucoprotease.	12 dys.	0.09	0.16
Pneumococci in salt solution.	12 dys.	0.08	0.00

The exact chemical changes that occur in this experiment are still under investigation, but it can be definitely concluded from the gross and microscopic appearances, as well as from the titrations cited, that leucoprotease causes a more extensive digestion of pneumococci than occurs when the organisms are suspended in salt solution. Intracardiac injections into guinea pigs showed that the products of leucoprotease digestion were certainly less toxic than the products of autolysis, and we assumed that probably leucoprotease carried proteolysis of pneumococci beyond the toxic stage.

Rosenow's interesting studies, which were published while this

work was in preparation, show a direct parallelism. He proved that autolysis of pneumococci resulted first in producing a toxic substance, which when injected directly into the circulation was capable of killing guinea pigs with all the symptoms of anaphylactic shock. When digestion was carried further, the toxic substance disappeared. Rosenow considers it probable that the toxic substance "is an earlier split product of pneumococci than the peptone stage." Our previous work showed that leucoprotease split mainly to albumose and to a slight extent to peptone, which affords support to Rosenow's assumption. The toxic substance then must be closely related to the protein molecule, and the substances resulting from leucoprotease digestion are deeper proteolytic cleavage products.

We then attempted to study what effect the injection of these various digestion products would produce in rabbits.

Ten rabbits were used. Rabbit 1 was given pneumococci heated to 60° C. in salt solution, centrifuged, and washed. The supernatant fluid was divided in half and injected into rabbits 2 and 3. Rabbits 4 and 5 were given the supernatant fluid from pneumococci treated with leucoprotease, and rabbits 6 and 7 similar fluid from pneumococci autolyzed in salt solution. Rabbit 8 was immunized in the usual way by one injection of dead organisms followed by several injections of live pneumococci. Rabbit 9 received the washed sediment from the pneumococci treated with leucoprotease, and rabbit 10 the similar sediment from the autolyzed pneumococci.

This long series was planned in order to get standards of immunity, and the details of each injection will be given solely for those animals in which we are especially interested. The general method of procedure was to use twenty-four hour broth cultures which were centrifuged and washed. The organisms were then suspended in salt solution and halved. To one half a certain amount, generally two cubic centimeters, 0.016 of a gram of leucoprotease was added, and to the other the same amount of salt solution. Chloroform was then added and the flasks were incubated at 37° C.

The time of incubation varied, and will be given in each protocol. Before injection the mixtures were thoroughly centrifuged at a speed of 3,000 revolutions per minute for from one half to one hour, the supernatant fluid and the chloroform were removed, and the sediment was washed in salt solution. The same amount of

fluid was always used, i. e., the salt autolysate injection represented exactly the same amount of pneumococci as did the leucoprotease.

Rabbit A 4.—Injected with the supernatant fluid from the digestion of pneumococci by leucoprotease.

Date of injection.	Incubation period of pneumococci and leucoprotease.	Method of injection.
Jan. 30, 1912.	14 dys.	Intraperitoneal.
Feb. 5, 1912.	6 dys.	Intraperitoneal.
Feb. 23, 1912.	6 dys.	Subcutaneous.
Mar. 3, 1912.	6 dys.	Subcutaneous.
Mar. 13, 1912.	5 dys.	Subcutaneous.

Rabbit A 6.—Injected with the supernatant fluid from the autolysis of pneumococci in salt solution. Protocol identical with that of rabbit A 4.

Rabbit A 9.—Injected with the washed sediment from digestion of pneumococci by leucoprotease. Protocol identical with that of rabbit A 4.

Rabbit A 10.—Injected with the washed sediment from autolysis of pneumococci in salt solution. Protocol identical with that of rabbit A 4.

The organisms used in these experiments were only moderately virulent and showed some spontaneous phagocytosis.

The determinations of both qualitative and quantitative immunity were made along several lines, but for the present the opsonic power of the serum will be used as a standard. In the present state of our knowledge, information gained by opsonic titrations must still be considered the most valuable comparative knowledge obtainable, for as yet sufficient data are not at hand to justify the application of newer methods, as, for instance, the measurement of the proteolytic power of the serum. However, we have made sufficient study of the newer phases of immunity to feel that shortly we shall be able to report more fully on these questions in pneumococcus immunity.

On March 9 fresh unheated sera were tested for opsonins against the homologous organism, by the Neufeld technique. Rabbit A 4, which had received the products of digestion by leucoprotease showed a well marked high grade of opsonic immunity. In the serum from rabbit A 6, which had received the salt autolysate, no trace of opsonic immunity could be shown. Rabbit A 9, which had received the sediment from the action of leucoprotease on pneumococci, likewise showed no immunity, whereas rabbit A 10, which

had received the bacterial bodies remaining after autolysis, gave a high degree.

On March 26 this experiment was repeated and verified with sera seventeen days old. We attempted to demonstrate opsonic immunity against two heterologous strains, one highly virulent and one avirulent, and in both experiments we found no increase in any serum. These results correspond with those obtained for opsonins by the usual method of injection, but whether the specificity of opsonic immunity is an actual index of the true immunity is doubtful. Several attempts to demonstrate complement-binding amboceptors in these sera were unsuccessful, even though we used as antigen suspensions of the organisms in salt solution, as well as watery and alcoholic extracts.

In series B the same general method was employed.

Rabbit B 4.—Injected with the supernatant fluid from the digestion of pneumococci by leucoprotease.

Date of injection.	Incubation period of pneumococci and leucoprotease.	Method of injection.
Mar. 23, 1912.	8 dys.	Subcutaneous.
Apr. 9, 1912.	13 dys	Subcutaneous.
Apr. 22, 1912.	5 dys.	Subcutaneous.
May 13, 1912.	2 dys.	Subcutaneous.
May 31, 1912.	3 dys.	Subcutaneous
June 8, 1912.	4 dys.	Subcutaneous.

Rabbits B 6, B 9, and B 10 correspond with the animals in series A.

Rabbit B 6.—Injected with the supernatant fluid from the autolysis of pneumococci in salt solution. Protocol identical with that of rabbit B 4.

Rabbit B 9.—Injected with the washed sediment from digestion of pneumococci by leucoprotease. Protocol identical with that of rabbit B 4.

Rabbit B 10.—Injected with the washed sediment from autolysis of pneumococci in salt solution. Protocol identical with that of rabbit B 4.

The strain of organisms used in these experiments was highly virulent and had resisted phagocytosis by the sera of all the animals before injections were begun.

On June 18 the sera of all animals were tested for opsonic immunity by the Neufeld technique. Sera had been drawn on June 8 and had been kept on ice without preservatives. Here we were again able to demonstrate a well marked high grade of opsonic immunity in the rabbit treated with the cleavage products of leuco-

protease on pneumococci. In this experiment, as in the one previously outlined, the quantitative estimation of immune opsonins showed that the highest grade of immunity had been reached by the rabbit receiving the products of leucoprotease digestion; and again here the immunity was found to exist only for the homologous strain of pneumococci. Complement-binding amboceptors could not be demonstrated.

In a third series of experiments we were able to verify in every detail the results already outlined.

SUMMARY.

1. Leucoprotease splits pneumococci into lower cleavage products than are obtained with the autolytic ferment contained in the pneumococci.

2. Such cleavage products injected into rabbits have the property of producing immune bodies in the blood of the injected animals.

3. These experiments indicate that proteolysis plays a part in the production of pneumococcus immunity.

BIBLIOGRAPHY.[2]

1. Wadsworth, A., *Jour. Exper. Med.*, 1912, xvi, 54.
2. Strouse, S., *Jour. Exper. Med.*, 1911, xiv, 109.
3. Lamar, R. V., *Jour. Exper. Med.*, 1911, xiii, 1.
4. Rosenow, E. C., *Jour. Infect. Dis.*, 1911, ix, 190.
5. Rosenow, E. C., *Jour. Infect. Dis.*, 1912, x, 113.
6. Rosenow, E. C., *Jour. Infect. Dis.*, 1912, xi, 94.
7. Dick, G. F., *Jour. Infect. Dis.*, 1912, x, 383.
8. Jobling, J. W., and Strouse, S., *Jour. Exper. Med.*, 1912, xvi, 269.

[2] Since this article went to press, Hirschfelder (*Jour. Am. Med. Assn.*, 1912, lix, 1373) has published a communication on the effect of pancreatin on pneumococci. Broth culture organisms were treated for fifteen minutes at 37° C., were then acidified and passed through a Pasteur filter. The "soluble vaccine" which results from this treatment has the property of preventing pneumococcus infections in susceptible animals, and the serum of these animals also has the property of preventing pneumococcus infections. The vaccine was used in a small series of cases of lobar pneumonia in man with apparently favorable results.

INDEX TO VOLUME XVI.

ADRENALIN in blood, 502
Anaphylaxis and retention of foreign protein by the kidney, 349
Anemia, post-hemorrhagic, 325
Anesthesia produced by the intravenous injection of ether, glycosuria following, 178
Antigenic properties of a protein compounded with casein, 479
Antigenic properties of split products of casein, 470
Antigens from spirochæta cultures, complement fixation in syphilis with, 336
Antipneumococcus serum, univalent, susceptibility of pneumococcus strains to, 680
Antitoxin, diphtheria, preventive action of, in serum-sensitized rabbits, 216
Antityphoid inoculation, immune bodies in the blood after, 315
Aorta, thoracic, permanent intubation of, 17
Arteries, influence of isoagglutinins on the final results of homoplastic transplantations of, 169
Artery, coronary, action of epinephrin on, 532
Auricles, fibrillation of, effects upon the circulation of, 395
AUSTIN, J. H. See PEARCE and AUSTIN, 780
——. See PEARCE, AUSTIN, and EISENBREY, 375
——. See PEARCE, AUSTIN, and KRUMBHAAR, 363
——. See PEARCE, AUSTIN, and MUSSER, 758
Autolysis, pigment formation in the liver during, 607

BACHMANN, GEORGE. A physiologico-pathological study of a case of heart-block occurring in a dog as a result of natural causes, 25
Bacilli, tubercle, human and bovine types of, in bone and joint tuberculosis in children, 432
Bacterial strains, closely related, selective action of gentian violet on, 822
Bactericidal action, selective, of gentian violet, 221
Bactericidal substance in leucocytic extract, nature of, 249
BASS, C. C., and JOHNS, FOSTER M. The cultivation of malarial plasmodia (*Plasmodium vivax* and *Plasmodium falciparum*) *in vitro*, 567
Blood, carbon dioxide content of, in pneumonia, 701
Blood, changes in, after splenectomy, and their relation to hemolytic jaundice, 758
Blood, circulating, occurrence and virulence of pneumococci in, during lobar pneumonia, 680
Blood, coagulation time of, in lobar pneumonia, 693
Blood corpuscles, red, increased resistance of, after splenectomy, 769
Blood destruction and regeneration and hemolytic jaundice, relation of the spleen to, 363, 375, 758, 769, 780
Blood, epinephrin (adrenalin) in, 502
Blood, immune bodies in, after antityphoid inoculation, 315
Blood, nitrogen retention in, in experimental acute nephritis of the cat, 789
Blood pressure, relation of epinephrin to, in the circulation, 541
Blood serum and plasma, vasoconstrictor and vasodilator properties of, 103
Blood serum, chemistry of, 719
Bone and joint tuberculosis in children, human and bovine tubercle bacilli in, 432
Bronchi, physiological action of epinephrin on the, 558
Bronchopneumonia, experimental, by intrabronchial insufflation, 126
BULL, CARROLL G. See JOBLING and BULL, 483
BULLOCK, F. D., and ROHDENBURG, G. L. Cell proliferation and parasites in rats, 527

CALCIFICATION, relation to, of lipoid metabolism in the developing chick, 512

Cancer, human, analogy between tumors of white mice and white rats and, 155
Cantharidin, experimental nephritis produced by, 831
Carbon dioxide content of blood in pneumonia, 701
CARREL, ALEXIS. Permanent intubation of the thoracic aorta, 17
——. Pure cultures of cells, 165
Casein, antigenic properties of a protein compounded with, 479
Casein, antigenic properties of split products of, 470
CECIL, RUSSELL L. The effect of certain experimental procedures on the islands of Langerhans, 1
Cell proliferation and parasites in rats, 527
Cells, endothelial, of lymph nodes and liver, changes in, in splenectomized animals receiving hemolytic serum, 780
Cells, pure cultures of, 165
Chemistry of blood serum, 719
Chemo-immunological studies on localized infections. Fourth paper: Experimental pneumococcic meningitis and its specific treatment, 581
CHURCHMAN, JOHN W. The selective bactericidal action of gentian violet, 221
CHURCHMAN, JOHN W., and MICHAEL, W. HOWARD. The selective action of gentian violet on closely related bacterial strains, 822
Circulation, effects of fibrillation of the auricles upon, 395
Circulation, epinephrin in the, and its relation to blood pressure, 541
CLARK, PAUL F. See HOWARD and CLARK, 850
Cleavage products, proteolytic, of pneumococci, immunization with, 860
Coagulation time of the blood in lobar pneumonia, 693
COFFEN, T. HOMER. See MEARA, COFFEN, and CREHORE, 280
COHN, ALFRED E. On the differences in the effects of stimulation of the two vagus nerves on rate and conduction of the dog's heart, 732
COLE, RUFUS. Toxic substances produced by pneumococcus, 644
COLWELL, H. S. See SPRUNT, COLWELL, and HAGAN, 607
Complement fixation in syphilis with spirochæta culture antigens, 336
Coronary artery, action of epinephrin on the, 532
Corpuscles, red blood, increased resistance of, after splenectomy, 769
CRAIG, CHARLES F., and NICHOLS, HENRY J. A study of complement fixation in syphilis with spirochæta culture antigens, 336
CRASTER, C. V. Conditions governing the growth of displaced normal tissue, 493
CREHORE, ALBERT C. See MEARA, COFFEN, and CREHORE, 280
Cultivation of malarial plasmodia *in vitro*, 567
Cultivation of *Spirochæta gallinarum*, 620
Cultivation of *Treponema pallidum* in fluid media, 211
Cultivation, pure, of *Spirochæta duttoni, Spirochæta kochi, Spirochæta obermeieri,* and *Spirochæta novyi*, 199
Cultivation, pure, of *Spirochæta phagedenis*, 261
Cultivation, pure, of *Treponema mucosum*, 194
Culture antigens, spirochæta, complement fixation in syphilis with, 336
Culture media, influence of, on the growth of tissue outside of the organism, 421
Cultures, pure, of cells, 165

DENIS, W. See FOLIN, KARSNER, and DENIS, 789
Diphtheria antitoxin, preventive action of, in serum-sensitized rabbits, 216
DIXON, ROBERT L. Some observations of the thoracic duct lymph after injection of oil of turpentine into the peritoneal cavity of the dog, 139
DOCHEZ, A. R. The presence of protective substances in human serum during lobar pneumonia, 665
——. The occurrence and virulence of pneumococci in the circulating blood during lobar pneumonia and the susceptibility of pneumococcus strains to univalent antipneumococcus serum, 680
——. Coagulation time of the blood in lobar pneumonia, 693
Duct, thoracic, lymph of, after injection of oil of turpentine into the peritoneal cavity of the dog, 139
Duttoni, Spirochæta, pure cultivation of, 199

EDEMA of the liver and lymph formation with experimental nephritis produced by cantharidin, 831

EISENBREY, A. B. See PEARCE, AUSTIN, and EISENBREY, 375
Electrocardiograph, study with the, of the mode of death of the human heart, 291
Endothelial cells of lymph nodes and liver, changes in, in splenectomized animals receiving hemolytic serum, 780
Epinephrin, action of, on the coronary artery, 532
Epinephrin in blood, 502
Epinephrin in the circulation and its relation to blood pressure, 541
Epinephrin, physiological action of, on the bronchi, 558
EPSTEIN, ALBERT A. A contribution to the study of the chemistry of blood serum, 719
ERLANGER, JOSEPH. Sinus stimulation as a factor in the resuscitation of the heart, 452
Ether, glycosuria following anesthesia produced by intravenous injection of, 178
Experimental acute nephritis of the cat, nitrogen retention in blood in, 789
Experimental bronchopneumonia by intrabronchial insufflation, 126
Experimental nephritis produced by cantharidin, lymph formation and edema of the liver with, 831
Experimental pneumococcic meningitis and its specific treatment, 581
Experimental procedures, effect of, on the islands of Langerhans, 1

FALCIPARUM, *Plasmodium*, cultivation of, *in vitro*, 567
Ferment action and a specific immune lipase, 483
Ferment action and immunization with proteolytic cleavage products of pneumococci, 860
Ferment action and leucocytic proteolysis, 269
Fibrillation of the auricles, effects upon the circulation of, 395
Fibrils, nerve, in the sino-auricular node, 613
Fluid media, cultivation of *Treponema pallidum* in, 211
FOLIN, OTTO, KARSNER, HOWARD T., and DENIS, W. Nitrogen retention in the blood in experimental acute nephritis of the cat, 789
FRASER, JOHN. The relative prevalence of human and bovine types of tubercle bacilli in bone and joint tuberculosis occurring in children, 432

GALLINARUM, *Spirochæta*, cultivation of, 620
Gaucher type of primary splenomegaly, 797
GAY, FREDERICK P., and ROBERTSON, T. BRAILSFORD. The antigenic properties of split products of casein, 470
——. The antigenic properties of a protein compounded with casein, 479
Gentian violet, selective action of, 221, 822
Glycosuria following anesthesia produced by the intravenous injection of ether, 178
Gout, nitrogen and nuclein metabolism in, 303

HAGAN, H. J. See SPRUNT, COLWELL, and HAGAN, 607
HANES, FREDERICK M. Lipoid metabolism in the developing chick and its relation to calcification, 512
HARVEY, SAMUEL C. See STEWART and HARVEY, 103
HAUPT, W. C. See KING, MOYLE, and HAUPT, 178
Heart, human, study with the electrocardiograph of the mode of death of, 291
Heart of the dog, differences in the effects of stimulation of the two vagus nerves on rate and conduction of the, 732
Heart, sinus stimulation as a factor in the resuscitation of the, 452
Heart-block, physiologico-pathological study of a case of, occurring in a dog as a result of natural causes, 25
Hemochromatosis, pigmentation of, relation of pigment formation in the liver during autolysis to, 607
Hemoglobinemia, relation of, to hemoglobinuria and jaundice in normal and splenectomized animals, 375
Hemoglobinuria and jaundice, relation of hemoglobinemia to, in normal and splenectomized animals, 375
Hemolytic jaundice and blood destruction and regeneration, relation of the spleen to, 363, 375, 758, 769, 780
Hemolytic jaundice, relation of changes in blood after splenectomy to, 758
Hemolytic serum, changes in endothelial cells of lymph nodes and liver in splenectomized animals receiving, 780

Hemolytic serum, reactions to, after splenectomy, 363
Homoplastic transplantations of arteries, influence of isoagglutinins on the final results of, 169
HOWARD, C. W., and CLARK, PAUL F. Experiments on insect transmission of the virus of poliomyelitis, 850

IMMUNE bodies in the blood after antityphoid inoculation, 315
Immune lipase, specific, and ferment action, 483
Immune sera, action of, on pneumococcus infection, 78
Immunization with proteolytic cleavage products of pneumococci, 860
Immunology, a study of increased resistance of red blood corpuscles after splenectomy, by the methods of, 769
In vitro cultivation of malarial plasmodia, 567
Infection, pneumococcus, action of immune sera on, 78
Infection, pneumococcus, in animals, 54, 78
Infections, localized, chemo-immunological studies on, 581
INGEBRIGTSEN, RAGNVALD. The influence of isoagglutinins on the final results of homoplastic transplantations of arteries, 169
——. Studies upon the characteristics of different culture media and their influence upon the growth of tissue outside of the organism, 421
Inoculation, antityphoid, immune bodies in the blood after, 315
Inoculation of tumors into organs, 155
Insect transmission of the virus of poliomyelitis, 850
Insufflation, intrabronchial, experimental bronchopneumonia by, 126
Intrabronchial insufflation, experimental bronchopneumonia by, 126
Intravenous injection of ether, glycosuria following anesthesia produced by, 178
Intubation, permanent, of the thoracic aorta, 17
Islands of Langerhans, effect of certain experimental procedures on, 1
Isoagglutinins, influence of, on the final results of homoplastic transplantations of arteries, 169

JANEWAY, THEODORE C., and PARK, EDWARDS A. The question of epinephrin in the circulation and its relation to blood pressure, 541
Jaundice and hemoglobinuria, relation of hemoglobinemia to, in normal and splenectomized animals, 375
Jaundice, hemolytic, and blood destruction and regeneration, relation of the spleen to, 363, 375, 758, 769, 780
Jaundice, hemolytic, relation of changes in blood after splenectomy to, 758
JOBLING, JAMES W., and STROUSE, SOLOMON. Studies on ferment action. II. The extent of leucocytic proteolysis, 269
JOBLING, JAMES W., and BULL, CARROLL G. Studies on ferment action. III. A specific immune lipase, 483
JOBLING, JAMES W., and STROUSE, SOLOMON. Studies on ferment action. V. Immunization with proteolytic cleavage products of pneumococci, 860
JOHNS, FOSTER M. See BASS and JOHNS, 567
Joint and bone tuberculosis in children, human and bovine tubercle bacilli in, 432

KARSNER, H. T., and PEARCE, R. M. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. IV. A study, by the methods of immunology, of the increased resistance of the red blood corpuscles after splenectomy, 769
KARSNER, H. T. See FOLIN, KARSNER, and DENIS, 789
Kidney, retention of foreign protein by the, 349
KING, JOHN H., MOYLE, R. D., and HAUPT, W. C. Studies in glycosuria. Second paper: Glycosuria following anesthesia produced by the intravenous injection of ether, 178
Kochi, Spirochæta, pure cultivation of, 199
KRISTELLER, LEO. See LEVENE and KRISTELLER, 303
KRUMBHAAR, E. B. See PEARCE, AUSTIN, and KRUMBHAAR, 363

LAMAR, RICHARD V. Chemo-immunological studies on localized infections. Fourth paper: Experimental pneumococcic meningitis and its specific treatment, 581
Langerhans, islands of, effects of certain experimental procedures on, 1
Lesions, acute vascular, in mice, following injections of pneumococci, 443

Lesions, phagedenic, *Spirochæta phagedenis* in, 261
Leucocytic extract, nature of the bactericidal substance in, 249
Leucocytic proteolysis and ferment action, 269
LEVENE, P. A., and KRISTELLER, LEO. Nitrogen and nuclein metabolism in gout, 303
LEVIN, ISAAC. Changes in the tissue surrounding a growing tumor and the significance of the "precancerous state," 149
——. Tumor inoculation into organs and the analogy between human cancer and the tumors of white mice and white rats, 155
LEWIS, PAUL A. The preventive action of diphtheria antitoxin in serum-sensitized rabbits, 216
LEWIS, THOMAS. Fibrillation of the auricles: its effects upon the circulation, 395
Lipase, specific immune, and ferment action, 483
Lipoid metabolism in the developing chick and its relation to calcification, 512
Liver and lymph nodes, changes in endothelial cells of, in splenectomized animals receiving hemolytic serum, 780
Liver, edema of, and lymph formation, with experimental nephritis produced by cantharidin, 831
Liver, pigment formation in, during autolysis, 607
Lobar pneumonia, coagulation time of blood in, 693
Lobar pneumonia, occurrence and virulence of pneumococci in the circulating blood during, 680
Lobar pneumonia, protective substances in human serum during, 665
LUETSCHER, J. A. See SPRUNT and LUETSCHER, 443
Lymph formation and edema of the liver with experimental nephritis produced by cantharidin, 831
Lymph nodes and liver, changes in endothelial cells of, in splenectomized animals receiving hemolytic serum, 780
Lymph, thoracic duct, after injection of oil of turpentine into the peritoneal cavity of the dog, 139

MALARIAL plasmodia, cultivation of, *in vitro*, 567
MANDLEBAUM, F. S. A contribution to the pathology of primary splenomegaly (Gaucher type), with the report of an autopsy on a male child four and one half years of age, 797
MANWARING, WILFRED H. The nature of the bactericidal substance in leucocytic extract, 249
MEARA, FRANK S., COFFEN, T. HOMER, and CREHORE, ALBERT C. A comparison of simultaneous polygraph and micrograph tracings, 280
Media, culture, influence of, on the growth of tissue outside of the organism, 421
Media, fluid, cultivation of *Treponema pallidum* in, 211
MELTZER, S. J. See WOLLSTEIN and MELTZER, 126
Meningitis, experimental pneumococcic, and its specific treatment, 581
Metabolism, lipoid, in the developing chick, and its relation to calcification, 512
Metabolism of nitrogen and nuclein in gout, 303
MICHAEL, W. HOWARD. See CHURCHMAN and MICHAEL, 822
Micrograph and polygraph tracings, simultaneous, comparison of, 280
MILNE, LINDSAY S. Post-hemorrhagic anemia, 325
MOYLE, R. D. See KING, MOYLE, and HAUPT, 178
Mucin-producing spirochæta, *Treponema mucosum*, in pure culture, 194
Mucosum, Treponema, in pure culture, 194
MUSSER, JOHN H., JR. See PEARCE, AUSTIN, and MUSSER, 758

NEPHRITIS, experimental acute, of the cat, nitrogen retention in blood in, 789
Nephritis, experimental, produced by cantharidin, 831
Nerve fibrils in the sino-auricular node, 613
Nerves, vagus, differences in the effects of stimulation of the, on rate and conduction of the dog's heart, 732
NICHOLS, HENRY J. See CRAIG and NICHOLS, 336
Nitrogen and nuclein metabolism in gout, 303
Nitrogen retention in blood in experimental acute nephritis of the cat, 789
Node, sino-auricular, nerve fibrils in, 613
Nodes, lymph, and liver, changes in

endothelial cells of, in splenectomized animals receiving hemolytic serum, 780
NOGUCHI, HIDEYO. *Treponema mucosum* (new species), a mucin-producing spirochæta from pyorrhea alveolaris, grown in pure culture, 194
——. The pure cultivation of *Spirochæta duttoni, Spirochæta kochi, Spirochæta obermeieri,* and *Spirochæta novyi,* 199
——. A method for cultivating *Treponema pallidum* in fluid media, 211
——. Pure cultivation of *Spirochæta phagedenis* (new species), a spiral organism found in phagedenic lesions on human external genitalia, 261
——. Cultivation of *Spirochæta gallinarum,* 620
Novyi, Spirochæta, pure cultivation of, 199
Nuclein and nitrogen metabolism in gout, 303

OBERMEIERI, *Spirochæta,* pure cultivation of, 199
OPIE, EUGENE L. Lymph formation and edema of the liver with experimental nephritis produced by cantharidin, 831
OPPENHEIMER, ADELE. See OPPENHEIMER and OPPENHEIMER, 613
OPPENHEIMER, B. S., and OPPENHEIMER, ADELE. Nerve fibrils in the sino-auricular node, 613

PALLIDUM, *Treponema,* cultivation of, in fluid media, 211
Parasites in rats and cell proliferation, 527
PARK, EDWARDS A. Observations with regard to the action of epinephrin on the coronary artery, 532
——. The physiological action of epinephrin on the bronchi, 558
——. See JANEWAY and PARK, 541
Pathology of primary splenomegaly (Gaucher type), 797
PEABODY, FRANCIS W. The carbon dioxide content of the blood in pneumonia, 701
PEARCE, RICHARD M. The retention of foreign protein by the kidney. A study in anaphylaxis, 349
PEARCE, RICHARD M., AUSTIN, J. H., and KRUMBHAAR, E. B. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. I. Reactions to hemolytic serum at various intervals after splenectomy, 363
PEARCE, RICHARD M., AUSTIN, J. H., and MUSSER, JOHN H., JR. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. II. The relation of hemoglobinemia to hemoglobinuria and jaundice in normal and splenectomized animals, 375
PEARCE, RICHARD M., AUSTIN, J. H., and MUSSER, J. H., JR. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. III. The changes in the blood following splenectomy and their relation to the production of hemolytic jaundice, 758
PEARCE, RICHARD M., and AUSTIN, J. H. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. V. Changes in the endothelial cells of the lymph nodes and liver in splenectomized animals receiving hemolytic serum, 780
PEARCE, RICHARD M. See KARSNER and PEARCE, 769
Peritoneal cavity of the dog, thoracic duct lymph after injection of oil of turpentine into, 139
Phagedenic lesions, *Spirochæta phagedenis* in, 261
Phagedenis, Spirochæta, pure cultivation of, 261
Pigment formation in the liver during autolysis and its relation to the pigmentation of hemochromatosis, 607
Pigmentation of hemochromatosis, relation to, of the pigment formation in the liver during autolysis, 607
Plasma and serum, comparison of, in testing for epinephrin in blood, 502
Plasma and serum of blood, vasodilator and vasoconstrictor properties of, 103
Plasmodia, malarial, cultivation of, *in vitro,* 567
Plasmodium falciparum, cultivation of, *in vitro,* 567
Plasmodium vivax, cultivation of, *in vitro,* 567
Pneumococcic meningitis, experimental, and its specific treatment, 581
Pneumococcus, acute vascular lesions in mice following injections of, 443
Pneumococcus, immunization with proteolytic cleavage products of, 860
Pneumococcus infection, action of immune sera on, 78

Pneumococcus infection in animals, 54, 78
Pneumococcus, occurrence and virulence of, in the circulating blood during lobar pneumonia, 680
Pneumococcus strains, susceptibility of, to univalent antipneumococcus serum, 680
Pneumococcus, toxic substances produced by, 644
Pneumonia, carbon dioxide content of blood in, 701
Pneumonia, lobar, coagulation time of blood in, 693
Pneumonia, lobar, occurrence and virulence of pneumococci in the circulating blood during, 680
Pneumonia, lobar, protective substances in human serum during, 665
Poliomyelitis, virus of, insect transmission of, 850
Polygraph and micrograph tracings, simultaneous, comparison of, 280
Post-hemorrhagic anemia, 325
Precancerous state, significance of, 149
Proliferation of cells, and parasites in rats, 527
Protein compounded with casein, antigenic properties of, 479
Protein, foreign, retention of, by the kidney, 349
Proteolysis, leucocytic, and ferment action, 269
Proteolytic cleavage products of pneumococci, immunization with, 860
Pyorrhea alveolaris, a mucin-producing spirochæta (*Treponema mucosum*) from, in pure culture, 194

RESUSCITATION of the heart, sinus stimulation as a factor in, 452
ROBERTSON, T. BRAILSFORD. See GAY and ROBERTSON, 470, 479
ROBINSON, G. CANBY. A study with the electrocardiograph of the mode of death of the human heart, 291
ROHDENBURG, G. L. See BULLOCK and ROHDENBURG, 527

SERA, immune, action of, on pneumococcus infection, 78
Serum and plasma, comparison of, in testing for epinephrin in blood, 502
Serum and plasma of blood, vasodilator and vasoconstrictor properties of, 103
Serum, hemolytic, changes in endothelial cells of lymph nodes and liver in splenectomized animals receiving, 780
Serum, hemolytic, reactions to, after splenectomy, 363
Serum, human, protective substances in, during lobar pneumonia, 665
Serum of blood, chemistry of, 719
Serum, univalent antipneumococcus, susceptibility of pneumococcus strains to, 680
Serum-sensitized rabbits, preventive action of diphtheria antitoxin in, 216
Sino-auricular node, nerve fibrils in, 613
Sinus stimulation as a factor in the resuscitation of the heart, 452
Spirochæta culture antigens, complement fixation in syphilis with, 336
Spirochæta duttoni, pure cultivation of, 199
Spirochæta gallinarum, cultivation of, 620
Spirochæta kochi, pure cultivation of, 199
Spirochæta, mucin-producing (*Treponema mucosum*), in pure culture, from pyorrhea alveolaris, 194
Spirochæta novyi, pure cultivation of, 199
Spirochæta obermeieri, pure cultivation of, 199
Spirochæta phagedenis, pure cultivation of, 261
Spleen, relation of, to blood destruction and regeneration and to hemolytic jaundice, 363, 375, 758, 769, 780
Splenectomized and normal animals, relation of hemoglobinemia to hemoglobinuria and jaundice in, 375
Splenectomized animals receiving hemolytic serum, changes in the endothelial cells of lymph nodes and liver in, 780
Splenectomy, changes in blood following, and their relation to hemolytic jaundice, 758
Splenectomy, increased resistance of red blood corpuscles after, 769
Splenectomy, reactions to hemolytic serum after, 363
Splenomegaly, primary, (Gaucher type), 797
SPRUNT, T. P., COLWELL, H. S., and HAGAN, H. J. Pigment formation in the liver during autolysis and its relation to the pigmentation of hemochromatosis, 607
SPRUNT, T. P., and LUETSCHER, J. A.

Acute vascular lesions in mice following injections of pneumococci, 443
STEWART, G. N. Testing for epinephrin (adrenalin) in blood. Comparison of plasma and serum, 502
STEWART, HUGH A., and HARVEY, SAMUEL C. The vasodilator and vasoconstrictor properties of blood serum and plasma, 103
STROUSE, SOLOMON. See JOBLING and STROUSE, 269, 860
Syphilis, complement fixation in, with spirochæta culture antigens, 336

THORACIC aorta, permanent intubation of, 17
Thoracic duct lymph after injection of oil of turpentine into the peritoneal cavity of the dog, 139
Tissue, changes in, surrounding a growing tumor, 149
Tissue, growth of, outside of the organism, influence of different culture media on, 421
Tissue, normal, displaced, conditions governing growth of, 493
Toxic substances produced by pneumococcus, 644
Tracings, simultaneous, of the polygraph and micrograph, comparison of, 280
Transplantation of tumor in mice, induced resistance toward, 629
Transplantations, homoplastic, of arteries, influence of isoagglutinins on the final results of, 169
Treponema mucosum in pure culture, 194
Treponema pallidum, cultivation of, in fluid media, 211
Tubercle bacilli, human and bovine, in bone and joint tuberculosis in children, 432
Tuberculosis, bone and joint, in children, human and bovine tubercle bacilli in, 432
Tumor, growing, changes in tissue surrounding, 149
Tumor inoculation into organs, 155
Tumor transplantation in mice, induced resistance toward, 629
Tumors of white mice and white rats, analogy between human cancer and, 155
Turpentine, oil of, thoracic duct lymph after injection of, into the peritoneal cavity of the dog, 139

VAGUS nerves, differences in the effects of stimulation of, on rate and conduction of the dog's heart, 732
Vascular lesions, acute, in mice following injections of pneumococci, 443
Vasoconstrictor and vasodilator properties of blood serum and plasma, 103
Vasodilator and vasoconstrictor properties of blood serum and plasma, 103
Violet, gentian, selective action of, 221, 822
Virus of poliomyelitis, insect transmission of, 850
Vivax, Plasmodium, cultivation of, *in vitro*, 567

WADSWORTH, AUGUSTUS B. Studies on pneumococcus infection in animals. First paper, 54
——. Studies on pneumococcus infection in animals. Second paper: Action of immune sera on pneumococcus infection, 78
WOGLOM, WILLIAM H. The duration and extent of induced resistance toward tumor transplantation in mice, 629
WOLLSTEIN, MARTHA. The duration of immune bodies in the blood after antityphoid inoculation, 315
WOLLSTEIN, MARTHA, and MELTZER, S. J. Experimental bronchopneumonia by intrabronchial insufflation, 126

v. 13. Folded parts.

GENERAL LIBRARY
DEC 2 - 1912
UNIV. OF MICH.

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY
SIMON FLEXNER, M.D.
AND
BENJAMIN T. TERRY, M.D.

VOLUME XVI, NO. 6. DECEMBER 1, 1912.

NEW YORK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter in the post office at Lancaster, Pa.

COPYRIGHT, 1912, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE NEW ERA PRINTING COMPANY
41 NORTH QUEEN STREET
LANCASTER, PA.

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

SIMON FLEXNER, M.D.
BENJAMIN T. TERRY, M.D. } *Editors*

The Journal of Experimental Medicine is published under the auspices of The Rockefeller Institute for Medical Research and is designed to cover the field of experimental medicine. It is a medium for the publication of investigations conducted in the Institute, or elsewhere under its grants; and it also accepts contributions of a suitable character from other sources.

MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Under the head of *Monographs of The Rockefeller Institute for Medical Research* are published from time to time scientific papers which are so extensive, or require such elaborate illustrations, as to render them unsuitable for current periodical issues.

Monographs will be sent post paid on application, at $1.00 each, payable in advance by check or money order.

Monograph No. 1.

(Issued June 30, 1910)

SIMON FLEXNER and J. W. JOBLING, Studies upon a Transplantable Rat Tumor. Plates 1–16.

J. W. JOBLING, The Biology of a Mixed Tumor of the Rat.

MAUD L. MENTEN, Experiments on the Influence of Radium Bromide on a Carcinomatous Tumor of the Rat.

J. W. JOBLING, Spontaneous Tumors of the Mouse. Plates 17–28.

J. W. JOBLING, Transplantation Experiments in *Macacus rhesus* with a Carcinomatous Teratoma from Man.

Monograph No. 2.

(Issued February 15, 1911)

WILLIAM TRAVIS HOWARD and OSCAR T. SCHULTZ, Studies in the Biology of Tumor Cells. Plates 1–6.

Monograph No. 3.

(Issued March 15, 1911)

BENJAMIN T. TERRY, Chemo-Therapeutic Trypanosome Studies with Special Reference to the Immunity Following Cure.

Monograph No. 4.

(Issued June 24, 1912)

FRANCIS W. PEABODY, GEORGE DRAPER, and A. R. DOCHEZ, A Clinical Study of Acute Poliomyelitis. Plates 1–13.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are assembled at irregular intervals and bound into volumes designated *Studies from The Rockefeller Institute for Medical Research*. A small number of the *Studies* are distributed free of charge to libraries, learned societies, and laboratories in which medical research is carried on. A few copies are reserved for sale, and may be obtained at $5.00 each.

For further information concerning the above publications application should be made to the

PUBLICATION DEPARTMENT
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
66TH STREET AND AVENUE A, NEW YORK

CONTENTS.

EPSTEIN, ALBERT A. A Contribution to the Study of the Chemistry of Blood Serum 719

COHN, ALFRED E. On the Differences in the Effects of Stimulation of the Two Vagus Nerves on Rate and Conduction of the Dog's Heart. PLATES 69–76 732

PEARCE, RICHARD M., AUSTIN, J. H., and MUSSER, JOHN H., JR. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice 758

KARSNER, H. T., and PEARCE, RICHARD M. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. IV. A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood Corpuscles after Splenectomy 769

PEARCE, RICHARD M., and AUSTIN, J. H. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. V. Changes in the Endothelial Cells of the Lymph Nodes and Liver in Splenectomized Animals Receiving Hemolytic Serum. PLATE 77 780

FOLIN, OTTO, KARSNER, HOWARD T., and DENIS, W. Nitrogen Retention in the Blood in Experimental Acute Nephritis of the Cat 789

MANDLEBAUM, F. S. A Contribution to the Pathology of Primary Splenomegaly (Gaucher Type), with the Report of an Autopsy on a Male Child Four and One Half Years of Age. PLATES 78–84 797

CHURCHMAN, JOHN W., and MICHAEL, W. HOWARD. The Selective Action of Gentian Violet on Closely Related Bacterial Strains. PLATE 85 822

OPIE, EUGENE L. Lymph Formation and Edema of the Liver with Experimental Nephritis Produced by Cantharidin. . 831

HOWARD, C. W., and CLARK, PAUL F. Experiments on Insect Transmission of the Virus of Poliomyelitis 850

JOBLING, JAMES W., and STROUSE, SOLOMON. Studies on Ferment Action. V. Immunization with Proteolytic Cleavage Products of Pneumococci 860

Index to Volume XVI 868

The Journal of Experimental Medicine is issued monthly, two volumes appearing in a year. Each volume will contain about six hundred pages and many plates and text-figures.

Papers for publication may be sent to the editors at The Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York. Other communications should be addressed to *The Journal of Experimental Medicine* in care of The Rockefeller Institute for Medical Research.

Contributors are requested to limit the length of articles submitted to *The Journal of Experimental Medicine* to twenty printed pages. Articles which exceed in length twenty-five printed pages will be returned to the authors in order that their contents may be reduced to this maximum, which does not include the space occupied by illustrations.

Authors receive fifty copies of their papers free; additional copies may be obtained at cost.

The subscription price is $5.00 (21 shillings, 21 marks, 26 francs) per year. The price of single numbers is $0.75. Subscriptions may be sent directly to *The Journal of Experimental Medicine*, 41 North Queen Street, Lancaster, Pa., or 66th Street and Avenue A, New York; or to The Macmillan Company, London; Gustav Fock, Leipzig; and Masson et Cie, Paris. Remittances sent direct to The Rockefeller Institute should be made by draft or check on New York, or by postal money order.

For information regarding complete sets and back numbers of *The Journal of Experimental Medicine*, application should be made to the Publication Department of The Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York.

C37